Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 7480–7493
Bioorganic & Medicinal Chemistry

Discovery of 1,1-dioxo-1,2,6-thiadiazine-5-carboxamide derivatives as cannabinoid-like molecules with agonist and antagonist activity

Carolina Cano,[a] Pilar Goya,[a] Juan Antonio Paez,[a,*] Rocío Girón,[b] Eva Sánchez[b] and María Isabel Martín[b]

[a]*Instituto de Química Médica, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain*
[b]*Universidad Rey Juan Carlos, Facultad C.C. de la Salud, Dpto. C.C. de la Salud III, Unidad de Farmacología, Avda. Atenas s/n, \E-28922 Alcorcón, Madrid, Spain*

Received 15 December 2006; revised 8 June 2007; accepted 6 July 2007
Available online 25 August 2007

Abstract—A series of new 2-substituted 1,1-dioxo-1,2,6-thiadiazine-5-carboxylate derivatives have been prepared from monosubstituted sulfamides in order to obtain *N*-substituted 1,1-dioxo-1,2,6-thiadiazine-5-carboxamides as novel cannabinoid derivatives, analogues of Rimonabant (SR141716A). Their potential functional activity on cannabinoid receptors has been evaluated in vitro and in vivo in mice, showing that two compounds (**37** and **39**) behave as cannabinoid agonists in vitro. Their potency is lower than that of the reference compound, WIN 55,212-2, but their efficacy is similar to that of this cannabinoid agonist, although no in vivo activity is observed. Another derivative (**38**) behaves as a cannabinoid antagonist both in vitro and in vivo, being its efficacy and potency similar to that of the well-known antagonist SR141716A.

1. Introduction

Several kinds of molecules interact with cannabinoid receptors including the classical cannabinoids such as Δ^9-tetrahydrocannabinol (Δ^9-THC)[1] that is the main psychotropic constituent of cannabis, their synthetic analogs,[2,3] the endogenous cannabinoids[4,5] and a series of diverse heterocyclic structures that have shown their ability to act like agonists or antagonists/inverse agonists.[6–10] Two types of cannabinoid receptors have so far been identified, CB_1 cloned in 1990[11] and CB_2 cloned in 1993.[12] The CB_1 cannabinoid receptor is found primarily in brain and neuronal tissue, whereas CB_2 is found mainly in immune cells where they may mediate an immunosuppressant effect. At present, there are some pharmacological evidences that support the existence of additional types or subtypes of cannabinoid receptors[13,14] and even two patents[15,16] claim that the G-protein-coupled receptor GPR55 is a novel cannabinoid receptor. Both cannabinoid receptor types belong to the large family of G-protein-coupled receptors (GPCRs) controlling a wide variety of signal transduction. The experimental three-dimensional structure of cannabinoid receptors have not yet been solved. However, different models of both CB_1 and CB_2 human receptors have been established by homology modelling using as template the X-ray structure of bovine Rhodopsin.[17,18] The interest in the potential medicinal properties claimed for cannabinoids include attenuation of nausea and vomiting in cancer chemotherapy, management of glaucoma, suppression of muscle spasticity/spasm associated with multiple sclerosis and spinal cord injury, disorders in neurobiology and analgesic effectiveness.[19–23] Cannabinoid receptor antagonists show potential therapeutic application as appetite suppressants, thus SR141716A (*N*-(piperidin-1-yl)-1-(2,4-dichlorophenyl)-5-(4-chlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide) has been introduced as an anti-obesity drug (Rimonabant; Acomplia®).

In an effort to search novel cannabinoids and taking into account different physico-chemical and biological studies of heterocycles containing the 1,2,6-thiadiazine system, new carboxamides of 1,1-dioxo-1,2-dihydro-1^{2,6}-1,2,6-thiadiazine derivatives have been designed. The similarity between 1,2,6-thiadiazine 1,1-dioxide and pyrazole has been described by comparison of their chemical and structural properties and thus, it has been

Keywords: Cannabinoid; 1,2,6-Thiadiazine; Cannabinoid agonist; Cannabinoid antagonist.
* Corresponding author. Tel.: +34 915622900; fax: +34 915644853; e-mail: juan@suricata.iqm.csic.es

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.07.056

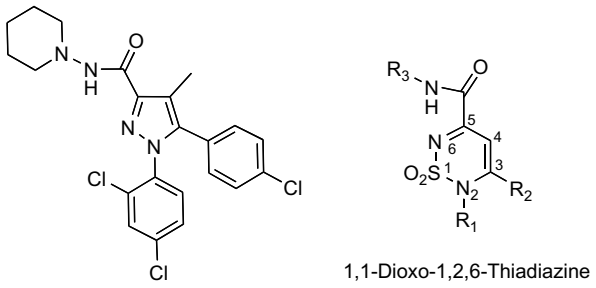


Figure 1. 1,1-Dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine carboxamides as analogues of SR141716A.

established that although there is difference in aromaticity, both series of heterocycles show similarities in the tautomeric equilibrium, the chemical shifts, and the reactivity of the 4-position[24] (Fig. 1).

The chemical structures of the compounds that interact with the cannabinoid receptors are very different and include some kinds of heterocyclic compounds such as benzopyranes, indoles, pyrroles, pyrazoles, imidazoles, triazoles, quinones and naphthyridines.[9] However, to date, 1,2,6-thiadiazine derivatives with cannabinoid properties have not yet been published.

Within this context, the purpose of this study has been the design and synthesis of a set of compounds based on the analogy between the pyrazole core of SR141716A (*N*-(piperidin-1-yl)-1-(2,4-dichlorophenyl)-5-(4-chlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide) and the 1,2,6-thiadiazine heterocyclic system focusing on the discovery of new cannabinoid ligands. The biological activity of the new synthesized compounds has been evaluated carrying out pharmacological experiments in the isolated tissue mouse vas deferens (MVD)[25] in order to determine if they behave as agonists or antagonists on cannabinoid receptors. Once they have been characterized by its functional activity as agonists or antagonists, then, compounds showing interesting profiles were again evaluated using the classic cannabinoid tetrad to test if they are able to in vivo reproduce the cannabinoid activity.[26,27]

The effect of the new compounds was always compared with that of reference drugs: WIN 55,212-2 as cannabinoid agonist and SR141716A as cannabinoid antagonist.

2. Result and discussion

2.1. Chemistry

The new 2-substituted 5-carboxamide-1,1-dioxothiadiazine derivatives here reported have been claimed by us in a patent.[28] Some related carboxamide derivatives to these compounds correspond to 2-unsubstituted 1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-3-carboxamides and were reported in an article[29] and in a patent[30] in which their synthesis was described.

The general synthetic route for the formation of substituted 1,2,6-thiadiazine-5-carboxamides comprises two steps involving ring formation between the monosubstituted sulfamide with adequately functionalized 2,4-dioxocarboxylic acid ethyl ester, and subsequent formation of amides from acylation of the corresponding amines (Scheme 1).

The structure of the 1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxamide system was modified by variations of the substituents at three different positions in order to study the structure–activity relationships modulating the biological activity. In position 3 of the heterocycle, different groups like methyl or aromatic substituents were chosen. In position 2, a variety of groups as phenyl, 4-chlorophenyl, benzyl, 2,4-dichlorobenzyl, hexyl, or cyclohexyl were considered, and finally *N*-substituted amides and hydrazides were selected in position 5, according to SAR in pyrazole derivatives.[31,32]

The first attempts to synthesize 2-substituted 1,2,6-thiadiazine-5-carboxylates were carried out with ethyl 2,4-dioxovalerate **7**. Thus, the reaction of **7** with phenyl, 4-chlorophenyl, benzyl, cyclohexyl and hexylsulfamides **1**–**3**, **5**–**6** in ethanol or diglyme afforded the corresponding 3-methyl-1,2,6-thiadiazine-3-carboxylates **11**–**15**.

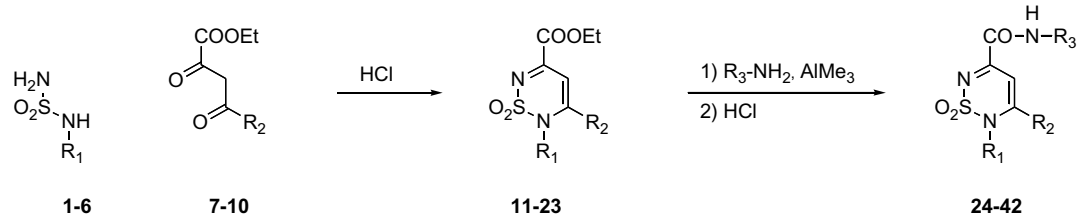
It is worth mentioning that only in the case of the preparation of 2-hexyl-3-methyl-5-carboxylic acid ester **15a** from the *N*-hexylsulfamide (**6**), the isomer 5-methyl-3-carboxylic acid ester **15b** was also isolated (Scheme 2).

The preparation of 4-bromophenyl **16**–**18**, 3-methyl-4-chlorophenyl **19**, **20** and 4-chlorophenyl-1,2,6-thiadiazine-3-carboxylates **21**–**23** were carried out by the reaction of the monosubstituted sulfamides **2**–**6** with the corresponding 2,4-dioxocarboxylates **8**–**10**.

The preparation of the hexyl and cyclohexyl sulfamides have been achieved using a general described procedure that consists in the reaction of sulfamide with the corresponding amines, in the appropriate refluxing solvent, depending on the amine solubility: in the case of *N*-benzylsulfamide[33] (**3**) and *N*-2,4-dichlorobenzylsulfamide[34] (**4**), in a solution of ethanol/water and for the and the *N*-cyclohexylsulfamide (**5**)[33] and *N*-hexylsulfamide (**6**)[35] in water (Scheme 3).

However, in the case of the reaction between aromatic amines with sulfamide, mixtures of mono and disubstituted sulfamides are obtained with this procedure. Therefore, another synthetic strategy for the preparation of *N*-phenylsulfamide[36] (**1**) and *N*-4-chlorophenylsulfamide[36,37] (**2**) was carried out by reaction of the corresponding aromatic amine and sulfamoyl chloride prepared from chlorosulphonyl isocyanate and formic acid.[36,37]

The preparation of 4-bromo **8**,[38] 4-chloro **9**[39] and 4-chloro-3-methyl **10**[40] 2,4-dioxocarboxylates have been carried out by a general described procedure.[38] Thus, reaction of the corresponding carbonylic compounds



1-6 **7-10** **11-23** **24-42**

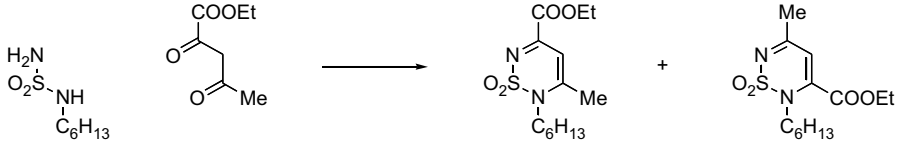
	R_1
1	Ph
2	4-ClPh
3	benzyl
4	2,4-DiClBn
5	cyclohexyl
6	hexyl

	R_2
7	Me
8	4-BrPh
9	4-ClPh
10	3-Me-4-ClPh

	R_1	R_2
11	Ph	Me
12	benzyl	Me
13	4-ClPh	Me
14	cyclohexyl	Me
15a	hexyl	Me
16	benzyl	4-BrPh
17	cyclohexyl	4-BrPh
18	2,4-DiClbenzyl	4-BrPh
19	benzyl	3-Me-4-ClPh
20	2,4-DiClBenzyl	3-Me-4-ClPh
21	benzyl	4-ClPh
22	2,4-DiClBenzyl	4-ClPh
23	hexyl	4-ClPh

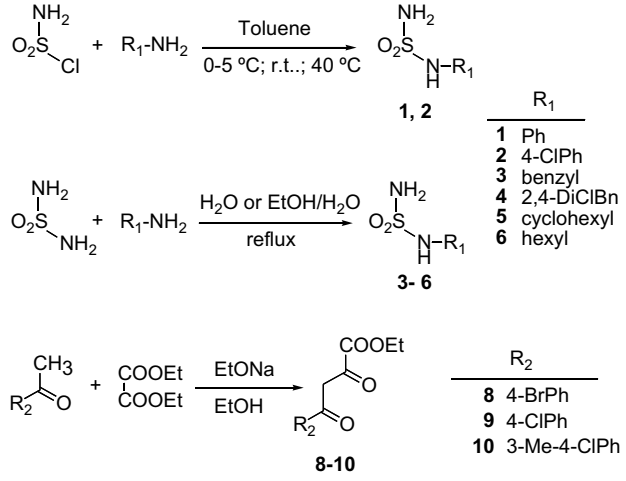
	R_1	R_2	R_3
24	Ph	Me	cyclohexyl
25	benzyl	Me	morpholinyl
26	benzyl	Me	piperidinyl
27	4-ClPh	Me	piperidinyl
28	cyclohexyl	Me	piperidinyl
29	hexyl	Me	piperidinyl
30	benzyl	4-BrPh	morpholinyl
31	benzyl	4-BrPh	piperidinyl
32	cyclohexyl	4-BrPh	piperidinyl
33	2,4-DiClbenzyl	4-BrPh	piperidinyl
34	benzyl	3-Me-4-ClPh	norbornyl
35	benzyl	3-Me-4-ClPh	piperidinyl
36	2,4-DiClbenzyl	3-Me-4-ClPh	cyclohexyl
37	benzyl	4-ClPh	Ph
38	benzyl	4-ClPh	piperidinyl
39	benzyl	4-ClPh	norbornyl
40	2,4-DiClbenzyl	4-ClPh	norbornyl
41	2,4-DiClbenzyl	4-ClPh	piperidinyl
42	hexyl	4-ClPh	piperidinyl

Scheme 1.



6 **7** **15a** **15b**

Scheme 2.



1, 2

	R_1
1	Ph
2	4-ClPh
3	benzyl
4	2,4-DiClBn
5	cyclohexyl
6	hexyl

3- 6

	R_2
8	4-BrPh
9	4-ClPh
10	3-Me-4-ClPh

8-10

Scheme 3.

and diethyl oxalate in ethanol and sodium ethoxide afforded the corresponding 2,4-dioxocarboxylic acid ethyl esters **8**–**10** (Scheme 3).

In the second step, formation of the amide, different methods can be used. The employed method consists in the acylation of the appropriate amines or hydrazines with carboxylic esters previously obtained. Thus, esters are treated with dimethyl aluminum amines or hydrazines, that are easily prepared from trimethyl aluminum and the corresponding amines or hydrazines to afford good yields of the corresponding hydrazides[41] or amides[42] under mild conditions.

The synthesis of *N*-piperidin-1-yl-5-carboxamide 3-methyl derivatives **26**–**29**, 4-bromophenyl **31**–**33**, 3-methyl-4-chlorophenyl **35** and 4-chlorophenyl **38**, **41**–**42** were carried out from **12**–**15**, **16**–**18**, **19** and **21**–**23** with *N*-aminopiperidine dimethylaluminum derivative.

The reaction of the 3-methyl or 3-(4-bromophenyl)-5-carboxylic esters **12** and **16** with *N*-aminomorpholine dimethylaluminum derivative afforded the morpholinyl derivatives **25** and **30**, respectively.

The preparation of the phenyl and cyclohexylamides **37** and **24** was carried out from the 3-(4-chlorophenyl) or

the 3-methylcarboxylic esters **21** and **11** with aniline and cyclohexylamine dimethylaluminum derivatives, respectively.

Finally, the preparation of the *N*-norbornyl-5-carboxamides **34**, **39**, **40** was carried out with the commercially available 1*R*,2*R*,4*S* and 1*R*,2*S*,4*S* mixture of the *N*-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl-amine and the corresponding ethyl esters **19**, **21**, **22** (Scheme 2).

The structures of all the new synthesized compounds have been established on the basis of their analytical and ^{13}C NMR and ^{1}H NMR spectroscopic data. The location of the carboxylic acid ethyl ester at position 3 or 5 was unequivocally established on the basis of the ^{13}C NMR chemical shifts at C-3 and C-5 and the multiplicity of the long range coupling constant. Thus, in the case of 5-carboxylic acid ester **15a**, the C-3 and C-5 signals appear at 161.3 and 162.7 ppm, whereas in the corresponding isomer **15b**, the signal corresponding to C-3 attached to the carboxylic group appears at higher field ($\delta = 145.2$) in relation to C-5 which appears at lower field ($\delta = 174.4$) (see Section 5). Moreover, derivative **12** was also unequivocally assigned as the 5-carboxylic acid ester isomer not only with the chemical shifts of C-3 and C-5 (162.4 and 162.5 ppm) but also by the observation of NOE effect/enhancement in the signal of the hydrogens of 3-methyl group and CH_2 of 2-benzyl group, only compatible with 5-carboxylic acid ester isomer.

3. Biology

3.1. Isolated tissues assays

The functional activity of the new compounds **24**–**42** has been tested on mouse vas deferens (MVD), a tissue commonly used to study and characterize cannabinoid effects.[25,43] This is a nerve-smooth muscle preparation that serves as a highly sensitive and quantitative functional in vitro bioassay for cannabinoid receptor agonists. Additionally, it is commonly used as a bioassay for competitive surmountable cannabinoid receptor antagonists and also provides a means for distinguishing neutral cannabinoid antagonists from inverse agonists. The bioassay of cannabinoid receptor agonists relies on the ability of these ligands to produce concentration-related decreases in the amplitude of electrically evoked contractions of the vas deferens. This they do by acting on naturally expressed prejunctional neuronal cannabinoid receptors to inhibit release of the contractile neurotransmitters, noradrenaline and ATP, that is provoked by the electrical stimulation. The bioassay of competitive surmountable cannabinoid receptor antagonists involves determining the ability of these compounds to produce dextral shifts in cannabinoid receptor agonist log concentration–response curves in electrically stimulated tissues.[44–46]

From the tested compounds **24**–**42**, at the tested concentrations (10^{-7}–2.4×10^{-5} M), some of them were able to modify the electrically induced contractile response on this tissue. As Figure 2 shows, compounds **29**, **32**, **36**, **37** and **39**, induced a dose-dependent inhibition of the electrically evoked contractions, reaching compounds **37** and **39** statistically significant effects as well as the reference cannabinoid receptor agonist used in this study, WIN 55,212-2 (10^{-7}–8.1×10^{-6} M). The EC_{50} of these compounds and the confidence limits are shown in Table 1.

From these data, it could be established that compounds **29**, **32**, **36**, **37** and **39** behave as agonists. Furthermore, the effectiveness (maximum effect) of compounds **37** and **39** was similar to that of the reference cannabinoid agonist WIN 55,212-2, although they were less potent (Fig. 2).

Considering that compounds **37** and **39** show an interesting profile as potential cannabinoid agonists, the antagonistic effect of SR141716A was tested. The partial reversion of the effect of these compounds by the cannabinoid antagonist (Fig. 3), indicates that their mechanism of action includes the activation of the cannabinoid receptor CB_1 confirming our hypothesis. Anyhow, more studies are needed to further determine if they also induce modifications through other non-CB_1 receptors.

Data obtained from other compounds (**24**–**28**, **30**, **31**, **33**–**35**, **38**, **40**–**42**), at the tested concentrations (10^{-7}–2.4×10^{-5} M) did not reveal any effect either on the non-stimulated basal recording or on the electrically in-

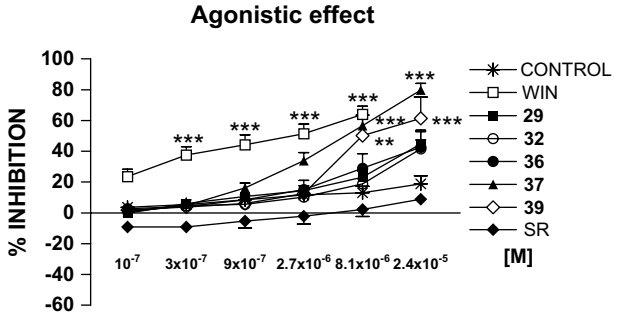


Figure 2. Lines show the mean % ± SEM ($n = 4$–7) of modification of the electrically induced contraction of the mouse vas deferens by addition of increasing concentrations of vehicle (CONTROL), WIN 55,212-2 (WIN), the new compounds **29**, **32**, **36**, **37** and **39** or SR141716A (SR). The * represents the significant difference versus control: $^{**}p < 0.01$, $^{***}p < 0.001$. (Two-way ANOVA test, Bonferroni's post-hoc test).

Table 1. EC_{50} and confidence limits for WIN 55,212-2 and new compounds

	EC_{50} (M)	Confidence limits (M)
WIN 55,212-2	1.45×10^{-6}	1.36×10^{-6}–1.54×10^{-6}
29	3.69×10^{-5}	3.21×10^{-5}–4.25×10^{-5}
32	3.84×10^{-5}	3.49×10^{-5}–4.21×10^{-5}
36	3.69×10^{-5}	3.53×10^{-5}–3.86×10^{-5}
37	5.73×10^{-6}	5.58×10^{-6}–5.89×10^{-6}
39	1.19×10^{-5}	1.05×10^{-5}–1.33×10^{-5}

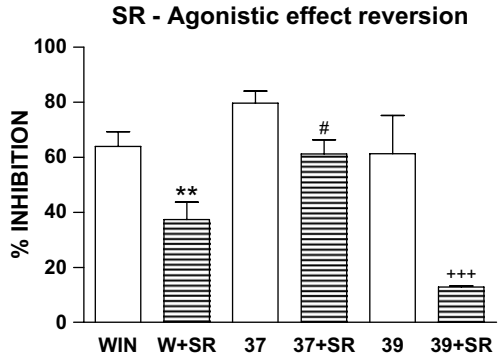


Figure 3. Reversion of the maximum agonistic effect of WIN 55,212-2 (WIN), **37** and **39** by the antagonist SR141716A (SR). White columns show the mean % ± SEM ($n = 4$–7) of inhibition of the electrically induced contraction of the mouse vas deferens induced by the addition of WIN (8.1×10^{-6} M) or the new compounds **37** and **39** (2.4×10^{-5} M) and the reversion of their inhibitory effect induced by the addition of SR141716A (SR) (striped columns). The ∗, # and + represent the significant difference versus WIN, **37** and **39** values, respectively: $^{**}p < 0.01$, $^{\#}p < 0.05$, $^{+++}p < 0.001$, Student's *t*-test (means comparison, unpaired *t*-test, two-tailed, confidence intervals 99%).

duced contractile response on MVD, so it could be established that they lack both direct (activation or blockade) or indirect (induction or inhibition of neurotransmitter release) intrinsic activity on a large number of well-known receptors that play a role in the basal tone or on the electrically induced contraction in this tissue (adrenergic, purinergic, cannabinoid, δ, μ and κ opioid receptors).

An interesting finding is that in the presence of the tested concentration (10^{-6} M) that lacks intrinsic activity, compounds **38** and **42**, significantly attenuated the inhibition induced by WIN 55,212-2 (Fig. 4) (**38**: $pA_2 = 6.92$, **42**: $pA_2 = 6.68$) as also occurred with the reference compound SR141716A ($pA_2 = 6.72$) (Table 2). These data suggest that **38** and **42** behave as antagonists as efficient as SR141716A and that they not display the inverse agonistic activity described for other cannabinoid CB_1

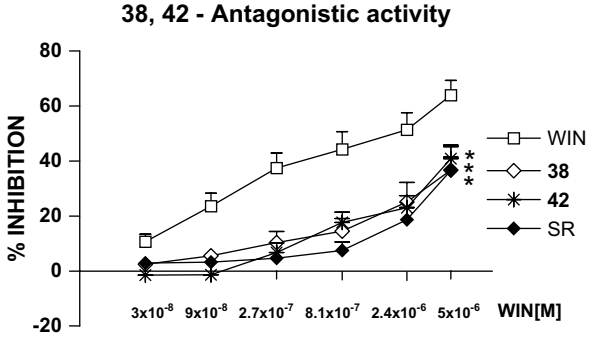


Figure 4. Lines show the mean % ± SEM ($n = 7$–8) inhibition of the electrically induced contraction of the mouse vas deferens induced by addition of increasing concentrations of WIN 55,212-2 in control tissues or in tissues incubated with compounds **38**, **42** or SR141716A (SR). The ∗ represents the significant difference versus control tissues: $^{*}p < 0.05$ (two-way ANOVA test, Bonferroni's post-hoc test).

Table 2. EC_{50} and confidence limits for WIN 55,212-2 in control group or in groups incubated with compounds **38**, **42** or SR141716A and their pA_2 calculated values

	EC_{50} (M)	Confidence limits (M)	pA_2
WIN 55,212-2	1.45×10^{-6}	1.36×10^{-6}–1.54×10^{-6}	
After **38**	$1.35 \times 10^{-5***}$	1.25×10^{-5}–1.46×10^{-5}	6.92
After **42**	$8.44 \times 10^{-6***}$	7.23×10^{-6}–9.87×10^{-6}	6.68
After SR141716A	$8.99 \times 10^{-6***}$	8.33×10^{-6}–9.69×10^{-6}	6.72

$^{***}p < 0.0001$ versus WIN 55,212-2 (unpaired Student's *t*-test, means comparison, two-tailed, confidence intervals 99%).

receptor antagonists.[7,8,47] Since they did not enhance the amplitude of electrically evoked contractions, they may be neutral cannabinoid CB_1 receptor antagonists. However, similar results have been obtained in the present and previous works after the administration of SR141716A, which is also accepted to be an inverse agonist.[48] Many other authors have also described that SR141716A exhibits greater potency in opposing effects induced by CB_1 agonists than in inducing inverse effects at CB_1 receptors by itself in isolated tissues.[49–53] So that, from present data cannabinoid inverse agonism could not be disregarded although the profile of these new compounds is interesting.

The fact that *N*-piperidin-1-yl-5-carboxamide derivates **38** and **42** behave as antagonist and **29** and **32** behave as agonists indicates that slight structural modifications in position 1 and 2 of the thiadiazine system greatly affect their pharmacological properties. This fact has also been shown in the case of pyrazole cannabinoid derivatives.[7,8]

3.2. In vivo assays

To further explore the cannabinoid pharmacological properties of the three compounds that display best profiles as agonists (**37**, **39**) or antagonists (**38**), their in vivo properties have been characterised on the basis of some of their behavioural effects in mice, using a well accepted model consisting of a series of four assays (cannabinoid tetrad): rectal temperature, catalepsy tested on an elevated ring, acute analgesia tested on a hot plate and spontaneous activity in an open field.[54,55]

Whereas intraperitoneal (ip) administration of the cannabinoid agonist WIN 55,212-2 (1.5 mg/kg) induced antinociception, hypothermia, inhibition of spontaneous motility and catalepsy, compounds **37** (10 and 20 mg/kg) and **39** (10 mg/kg) ip administered were not able to significantly modify any of the tested parameters, discarding in vivo activity at these doses. Treatment with **39** at 20 mg/kg has led to an increase in the locomotor activity without significantly affecting the other three parameters.

To disregard that the absence of activity may be attributed to low concentrations in the central nervous system, drugs have also been intracerebroventricularly (icv) injected. Results obtained failed to induce significant in vivo activity. Given by this way, whereas **39** (0.2 mg/kg) did not produce any cannabinoid behavioral

sign, derivative **37** at two (0.2 and 0.8 mg/kg) of the three tested doses (0.2, 0.4 and 0.8 mg/kg), just led to a certain degree of immobility at the ring test. As expected, WIN 55,212-2 (0.1, 0.2 and 0.4 mg/kg) was also dose-dependent active when icv administered (Table S2, supporting information).

More work is required to justify these in vivo and in vitro differences, especially the absence of significant in vivo activity of compounds **37** and **39** even when they were icv administered. In our opinion, these unexpected and in a way disappointing results could be, at least in part, attributed to the low solubility of these compounds which complicate their handling in the in vivo tests and perhaps may disturb their distribution and their access to the receptor atmosphere.

Compound **38**, both injected ip (1 mg/kg) and icv (0.05 mg/kg), according to the in vitro results, did not induce behavioral modifications, ruling out cannabinoid agonism. When its antagonistic activity was in vivo tested, results reached after its ip administration (1, 5 and 10 mg/kg) were unequal: it was able to block the antinociception induced WIN 55,212-2, and the smaller dose (1 mg/kg) prevented the reduction of the spontaneous activity. This partial although significant antagonizing effect of compound **38** is in agreement with those demonstrated in the in vitro experiments. Nevertheless, when compound **38** was icv given (0.1 mg/kg), it was able to antagonize all of the four cannabinoid signs induced by WIN 55,212-2. The reference CB_1 antagonist SR141716A ip administered antagonized, as expected, the effects of WIN 55,212-2 (Table S3, supporting information).

4. Conclusions

From a synthetic point of view, new 5-carboxamide derivatives of 1,1-dioxo-1,2,6-thiadiazine-5-carboxamide substituted in the 2-position have been prepared.

Regarding their pharmacological activity, compounds **37** and **39** have shown cannabinoid activity as cannabinoid agonists with similar effectiveness than that of the reference compound although their low solubility has probably limited their in vivo activity. Another new compound (**38**) with a profile of cannabinoid antagonist and with in vitro and in vivo efficacy has been described.

In conclusion, in this paper a novel heterocyclic system with versatile cannabinoid properties has been identified and reported here for the first time and thus, some 1,2,6-thiadiazine derivatives here described can be considered as new lead compounds.

5. Experimental

5.1. Chemistry

^1H and ^{13}C NMR spectra were recorded on Varian Unity 300 and 400 Varian Gemini and Bruker Avance 300 spectrometers. Chemical shifts are reported in ppm on the δ scale. The signal of the solvent was used as reference. Mass spectra (electrospray ionization) were determined on a MSD-Serie 1100 Hewlett Packard instrument. Melting points were determined on a Reichert Jung Thermovar melting point apparatus and are uncorrected. Elemental analyses were performed on a Heraeus CHN-O Rapid Analysis in our Analytical Services at Centro de Química Orgánica 'Manuel Lora Tamayo' (CSIC). Flash column chromatography was carried out by using Merck silica gel 60 (0.040–0.063). All starting materials were commercially available in Aldrich or Fluka and used without further purification, *N*-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl-amine hydrochloride was purchased from Maybridge. The monosubstituted sulfamides **1**–**4** were synthesized following the procedure of Paquin and the aromatic sulfamides **5** and **6** were prepared following the procedure of Lee. The ethyl 2,4-dioxovalerate **7** was purchased from Aldrich and the 2,4-dioxocarboxylic acid ethyl ester **8**–**10** were synthesized in the laboratory by the reaction of the corresponding ketone with diethyloxalate.

5.2. General procedure for the synthesis of 1,2,6-thiadiazine-5-carboxylic acid ethyl ester derivatives 11–23

A solution of the substituted sulfamide (4 mmol) and the corresponding 2,4-dioxocarboxylic acid ethyl ester (4 mmol) in dry diethylene glycol dimethyl ether (diglyme) or ethanol (50 mL) was saturated with a slow stream of hydrogen chloride and then refluxed for 30 h. The reaction mixture was evaporated to dryness and the residue was purified by silica gel column chromatography with CH_2Cl_2 as eluent to give the corresponding 1,2,6-thiadiazine derivative.

5.2.1. 2-Phenyl-3-methyl-1,1-dioxide-1,2,6-thiadiazine-5-carboxylic acid ethyl ester (11). From ethyl 2,4-dioxovalerate **7** (0.29 mL, $d = 1.126$, 2.0 mmol), *N*-phenylsulfamide **1** (0.344 g, 2.0 mmol) and diglyme (50 mL); reaction time 30 h. To the reaction mixture water was added and the precipitate was filtered yielding **14** (0.120 g, 66%); mp = 136–138 °C; ^1H NMR (500 MHz, $CDCl_3$) δ: 7.53–7.50 (m, 2H, *H*-2 and (4)Ph); 7.40 (m, 2H, *H*-3Ph); 6.61 (s, 1H, *H*-4); 4.41 (q, 2H, $J = 7.1$ Hz, CH_2(Et)); 2.07 (s, 3H, CH_3(4)); 1.40 (t, 3H, $J = 7.1$ Hz, CH_3(Et)). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.5 (C-5); 162.0 (C-3); 158.6 (CO); 133.4 (C-1Ph); 130.7 (C-4Ph); 130.1 (C-2Ph); 128.9 (C-3Ph); 100.3 (C-4); 63.2 (CH_2(Et)); 22.2 (CH_3(3)); 14.4 (CH_3(Et)). MS (ES+) $[M+H]^+$ 295 (100%). Anal. ($C_{13}H_{14}N_2O_4S$, 294.07): C, H, N, S.

5.2.2. 2-Benzyl-3-methyl-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxylic acid ethyl ester (12). From ethyl 2,4-dioxovalerate **7** (0.58 mL, $d = 1.126$, 4.0 mmol), *N*-benzylsulfamide **3** (0.750 g, 4.0 mmol) in diglyme (50 mL). Reaction time 30 h. Yield **12** (0.892 g, 72%); mp = oil; ^1H NMR (500 MHz, $CDCl_3$) δ: 7.24–7.16 (m, 5H, Ph); 6.45 (s, 1H, *H*-4); 5.05 (s, 2H, CH_2(Bn)); 4.30 (q, 2H, $J = 7.1$ Hz, CH_2(Et)); 2.15 (s, 3H, CH_3(3)); 1.30 (t, 3H, $J = 7.1$ Hz, CH_3(Et)). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.5 (C-5); 162.4 (C-3); 158.4 (CO); 134.0 (C-1Ph); 129.2 (C-4Ph); 128.8 (C-3Ph);

126.9 (C-2Ph); 102.3 (C-4); 63.2 (CH_2(Et)); 49.7 (CH_2(Ph)); 21.5 (CH_3(3)), 14.0 (CH_3(Et)). MS (ES+): $[M+H]^+$ 309 (100%). Anal. ($C_{14}H_{16}N_2O_4S$, 308.8): C, H, N, S.

5.2.3. 2-(4-Chlorophenyl)-3-methyl-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxylic acid ethyl ester (13). From ethyl 2,4-dioxovalerate **7** (0.465 mL, d = 1.126, 2.0 mmol), *N*-4-chlorophenylsulfamide **3** (0.413 g, 2.0 mmol) and dry diglyme (50 mL). Reaction time 24 h. Eluent CH_2Cl_2/MeOH/NH_3 (4/9/0.1). Yield **13** (0.037 g, 16%); mp = 146–148 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.48 (d, 2H, J = 8.8 Hz, *H*-3Ph); 7.35 (d, 2H, J = 8.8 Hz, *H*-2Ph); 6.61 (s, 1H, *H*-4); 4.41 (q, 2H, J = 7.3 Hz, CH_2(Et)); 2.07 (s, 3H, CH_3(3)); 1.40 (t, 3H, J = 7.3 Hz, CH_3(Et)). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.3 (C-5); 161.6 (C-3); 158.8 (CO); 137.6 (C-1Ph); 131.9 (C-4Ph); 130.4 (C-2Ph); 130.3 (C-3Ph); 100.5 (C-4); 63.3 (CH_2(Et)); 22.1 (CH_3(3)); 14.0 (CH_3(Et)). MS (ES+): $[M+H]^+$ 329 (100%); $[M+H+2]^+$ 331 (45%). Anal. ($C_{13}H_{13}ClN_2O_4S$, 328. 77): C, H, N, S.

5.2.4. 2-Cyclohexyl-3-methyl-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxylic acid ethyl ester (14). From ethyl 2,4-dioxovalerate **7** (0.29 mL, d = 1.126, 2.0 mmol), *N*-cyclohexylsulfamide **5**, (0.356 g, 2.0 mmol) in diglyme (40 mL). Reaction time 24 h. To the reaction mixture water was added and the precipitate was filtered yielding **14** (0.188 g, 31%); mp = 114–116 °C. MS (ES+) $[M+H]^+$ 301 (100%); H NMR (300 MHz, $CDCl_3$) δ: 6.46 (s, 1H, *H*-4); 4.37 (q, 2H, J = 7 Hz, CH_2(Et)); 4.09 (br m, 1H, *H*-1ax(Cy)); 2.41 (s, 3H, CH_3(3)); 2.33 (m, 2H, *H*-2eq(Cy)); 1.93 (m, 2H, *H*-2ax(Cy)); 1.92 (m, 2H, *H*-3ax(Cy)); 1.68 (m, 2H, *H*-4(Cy)); 1.37 (t, 3H, J = 7.0 Hz, CH_3(Et)); 1.27 (m, 2H, *H*-3eq(Cy)). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 162.7 (C-5); 161.5 (C-3); 157.7 (CO); 102.1 (C-4); 63.1 (CH_2(Et)); 62.4 (C-1Cy); 31.1 (C-2Cy); 26.4 (C-3Cy); 24.6 (C-4Cy); 22.5 (CH_3(3)); 14.0 (CH_3(Et)). Anal. ($C_{13}H_{20}N_2O_4S$, 300.37): C, H, N, S.

5.2.5. 2-Hexyl-3-methyl-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxylic acid ethyl ester (15a). From ethyl 2,4-dioxovalerate **7** (0.29 mL, d = 1.126, 2.0 mmol), *N*-hexylsulfamide **6** (0.361 g, 2.0 mmol) and dry diglime (30 mL). Reaction time 24 h. Yield **15a** (0.303 g of yellow oil, 50%); mp = oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.52 (s, 1H, *H*-4); 4.38 (q, 2H, J = 7.1 Hz, CH_2(Et)); 3.86 (m, 2H, *H*-1Hex); 2.40 (s, 3H, CH_3(3)); 1.81 (m, 2H, *H*-2Hex); 1.38 (t, 3H, J = 7.1 Hz, CH_3(Et)); 1.31 (m, 6H, *H*s Hex); 0.88 (m, 3H, CH_3(Hex)). Anal. ($C_{13}H_{13}ClN_2O_4S$, 328.77): C, H, N, S. ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.7 (C-5); 161.3 (C-3); 158.1 (CO); 101.8 (C-4); 63.1 (CH_2(Et)); 47.0 (C-1Hex); 31.2 (C-4Hex); 30.5 (C-2Hex); 26.3 (C-3Hex); 22.4 (C-5Hex); 21.0 (CH_3); 14.0 (CH_3(Et)); 13.9 (CH_3). MS (ES+) $[M+H]^+$ 303 (100%). Anal. ($C_{13}H_{22}N_2O_4S$, 302.39): C, H, N, S.

5.2.6. 2-Hexyl-5-methyl-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-3-carboxylic acid ethyl ester (15b). Compound **15b** was also isolated (0.017 g, 3%); mp = oil; MS (ES+) $[M+H]^+$ 303 (100%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.46 (s, 1H, *H*-4); 4.39 (q, 2H, J = 7.3 Hz, CH_2(Et)); 3.94 (m, 2H, *H*-1Hex); 2.37 (s, 3H, CH_3); 1.77 (m, 2H, *H*-2Hex); 1.40 (t, 3H, J = 7.3 Hz, CH_3(Et)); 1.28 (m, 6H, *H*s Hex); 0.87 (m, 3H, CH_3(Hex)). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 174.4 (C-5); 160.7 (CO); 145.2 (C-3); 107.4 (C-4); 63.4 (CH_2(Et)); 48.8 (C-1Hex); 31.1 (C-4Hex); 30.9 (C-2Hex); 26.1 (CH_3); 25.4 (C-3Hex); 22.4 (C-5Hex); 14.0 (CH_3(Et)); 13.9 (CH_3).

5.2.7. 2-Benzyl-3-(4-bromophenyl)-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxylic acid ethyl ester (16). From 4-(4-bromophenyl)-2,4-dioxobutyric acid ethyl ester **8** (0.233 g, 0.7 mmol), *N*-benzylsulfamide **3** (0.143 g, 0.7 mmol), diglyme (40 mL). Reaction time 24 h. Yield **16** (0.053 g, 33%); mp = 152–154 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.56 (d, 2H, J = 8.3 Hz, *H*-2Ph); 7.20 (m, 3H, *H*s Bn); 7.17 (d, 2H, J = 8.3 Hz, *H*-3Ph); 6.87 (d, 2H, J = 8.0 Hz, *H*-2Bn); 6.71 (s, 1H, *H*-5); 5.02 (s, 2H, CH_2(Bn)); 4.42 (q, 2H, J = 7.3 Hz, CH_2(Et)); 1.42 (t, 3H, J = 7.3 Hz, CH_3(Et)). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.3 (C-5); 161.0 (C-3); 159.8 (CO); 134.7 (C-1Bn); 132.4 (C-3Ph); 131.5 (C-4Ph); 129.8 (C-2Ph); 128.7 (C-3Bn); 128.3 (C-4Bn); 127.3 (C-2Bn); 126.7 (C-1Ph); 105.2 (C-4); 63.4 (CH_2(Et)); 14.0 (CH_3(Et)). MS (ES+) $[M+H]^+$ 449 (99%), $[M+H+2]^+$ 451 (100%). Anal. ($C_{19}H_{17}BrN_2O_4S$, 449.45): C, H, N, S.

5.2.8. 3-(4-Bromophenyl)-2-cyclohexyl-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxylic acid ethyl ester (17). From 4-(4-bromophenyl)-2,4-dioxobutyric acid ethyl ester **8** (0.120 g, 0.4 mmol), *N*-cyclohexylsulfamide **5** (0.072 g, 0.4 mmol) and dry EtOH (30 mL). Reaction time 24 h. Yield **17** (0.008 g, 5%); mp = 159–161 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.67 (d, 2H, J = 8.4 Hz, *H*-2Ph); 7.36 (d, 2H, J = 8.4 Hz, *H*-3Ph); 6.65 (s, 1H, *H*-5); 4.42 (q, 2H, J = 7.3 Hz, CH_2(Et)); 3.79 (tt, 1H, $J_{1ax-2ax}$ = 12.2 Hz; $J_{1ax-2eq}$ = 3.6 Hz, *H*-1ax(Cy)); 2.24 (m, 2H, *H*-2ax(Cy)); 2.00 (m, 1H, *H*-4eq(Cy)); 1.78 (m, 2H, *H*-2eq(Cy)); 1.39 (t, 3H, J = 7.3 Hz, CH_3(Et)); 0.95–1.34 (m, 3H, *H*-3 and *H*-4ax(Cy)). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.6 (C-5); 161.4 (C-3);159.8 (CO); 132.8 (C-1Ph); 132.7 (C-2Ph); 129.4 (C-3Ph); 126.5 (C-4Ph); 105.2 (C-4); 65.2 (C-1Cy); 63.3 (CH_2(Et)); 31.6 (C-2Cy); 26.1 (C-3Cy); 24.4 (C-4Cy); 14.0 (CH_3(Et)). MS (ES+) $[M+H]^+$ 441 (92%), $[M+H+2]^+$ 443 (100%). Anal. ($C_{18}H_{21}BrN_2O_4S$, 441.31): C, H, N, S.

5.2.9. 2-(2,4-Dichlorobenzyl)-3-(4-bromophenyl)-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxylic acid ethyl ester (18). From 4-(4-bromophenyl)-2,4-dioxobutyric acid ethyl ester **8** (0.351 g, 1.2 mmol), *N*-2,4-dichlorobenzylsulfamide **4** (0.300 g, 1.2 mmol) and dry ethanol (30 mL). Reaction time 72 h. Yield **18** (0.123 g, 20%); mp = oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.54 (d, 2H, J = 8.5 Hz, *H*-2Ph); 7.41–7.18 (m, 3H, *H*s Bn); 7.15 (d, 2H, J = 8.5 Hz, *H*-3Ph); 6.76 (s, 1H, *H*-4); 5.05 (s, 2H, CH_2(Bn)); 4.43 (q, 2H, J = 7.3 Hz, CH_2(Et)); 1.41 (t, 3H, J = 7.3 Hz, CH_3(Et)). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 162.2 (C-5); 161.2 (C-3); 160.2 (CO); 134.7 (C-1Bn); 133.0 (C-4Bn); 132.6 (C-3Ph);

131.3 (C-2Bn); 131.1 (C-4Ph); 129.5 (C-2Ph); 129.4 (C-3Bn); 129.3 (C-6Bn); 127.8 (C-5Bn); 127.1 (C-1Ph); 105.3 (C-4); 63.6 (CH_2(Et)); 49.4 (CH_2(Bn)); 14.0 (CH_3(Et)). MS (ES+) $[M+H]^+$ 519 (100%), 517 (62%), 521 (45%). Anal. ($C_{19}H_{15}BrCl_2N_2O_4S$, 518.21): C, H, N, S.

5.2.10. 2-Benzyl-3-(4-chloro-3-methylphenyl)-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxylic acid ethyl ester (19). From 4-(4-chloro-3-methylphenyl)-2,4-dioxobutyric acid ethyl ester **10** (0.336 g, 1.2 mmol) *N*-benzylsulfamide **3** (0.24 g, 1.3 mmol) and dry ethanol (40 mL). Reaction time 72 h. Yield **19** (0.152 g, 36%); mp = 109–111 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.42–6.91 (m, 8H, *H*sPh); 6.73 (s, 1H, *H*-4); 5.02 (s, 2H, CH_2(Bn)); 4.44 (q, 2H, J = 7.3 Hz, CH_2(Et)); 2.33 (s, 3H, CH_3-Ph); 1.43 (t, 3H, J = 7.3 Hz, CH_3(Et)). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 161.4 (C-3 and C-5); 159.8 (CO); 137.4 (C-4 (Ph)); 135.0 (C-1Bn); 130.9 (C-1Ph); 130.8 (C-2Ph); 129.7 (C-5Ph); 128.7 (C-3Bn); 128.2 (C-4Bn); 127.3 (C-2Bn); 126.9 (C-6Ph); 105.1 (C-4); 63.4 (CH_2(Et)); 52.3 (CH_2(Bn)); 19.9 (CH_3-Ph); 14.0 (CH_3(Et)). MS (ES+) $[M+H]^+$ 419 (100%), $[M+H+2]^+$ 421 (36%). Anal. ($C_{20}H_{19}ClN_2O_4S$, 418.89): C, H, N, S.

5.2.11. 2-(2,4-Dichlorobenzyl)-3-(4-chloro-3-methylphenyl)-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxylic acid ethyl ester (20). From 4-(4-chloro-3-methylphenyl)-2,4-dioxobutyric acid ethyl ester **10** (0.314 g, 1.2 mmol), *N*-2,4-dichlorobenzylsulfamide **4** (0.300 g, 1.2 mmol) and dry ethanol (30 mL). Reaction time 96 h. Yield **20** (0.041 g yellow oil, 7%); mp = oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.35 (d, 1H, J = 8.0 Hz, *H*-5Ph); 7.24 (m, 3H, *H*sBn); 7.10 (d, 1H, J = 2.2 Hz, *H*-2Ph); 7.04 (dd, 1H, J = 8.0 Hz, 2.2 Hz, *H*-6Ph); 6.76 (s, 1H, *H*-4); 5.05 (s, 2H, CH_2(Bn)); 4.43 (q, 2H, J = 7.3 Hz, CH_2(Et)); 2.30 (s, 3H, CH_3-Ph); 1.42 (t, 3H, J = 7.3 Hz, CH_3(Et)). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 162.2 (C-5); 161.6 (C-3); 160.2 (CO); 138.7 (C-3Ph); 137.7 (C-4Ph); 134.6 (C-1Bn); 132.9 (C-2Bn); 131.6 (C-1Ph); 130.6 (C-4Bn); 130.4 (C-2Ph); 130.0 (C-5Ph); 129.4 (C-6Bn); 129.3 (C-3Bn); 127.8 (C-5Bn); 126.6 (C-6Ph); 105.1 (C-4); 63.5 (CH_2(Et)); 49.5 (CH_2(Bn)); 20.1 (CH_3-Ph); 14.0 (CH_3(Et)). MS (ES+) $[M+H]^+$ 487 (100%), 489 (96%), 491 (37%). Anal. ($C_{20}H_{17}Cl_3N_2O_4S$, 487.78): C, H, N, S.

5.2.12. 2-Benzyl-3-(4-chlorophenyl)-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxylic acid ethyl ester (21). From 4-(4-chlorophenyl)-2,4-dioxobutyric acid ethyl ester **9** (0.094 g, 0.37 mmol) and *N*-benzylsulfamide **3** (0.069 g, 0.37 mmol). Reaction time 24 h. Yield **21** (0.028 g, 53%); mp = 135–137 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.39 (d, 2H, J = 8.5 Hz, *H*-2Ph); 7.20 (m, 3H, *H*s Bn); 7.24 (d, 2H, J = 8.5 Hz, *H*-3Ph); 6.87 (d, 2H, *H*-2Bn); 6.71 (s, 1H, *H*-4); 5.02 (s, 2H, CH_2(Bn)); 4.43 (q, 2H, J = 7.3 Hz, CH_2(Et)); 1.42 (t, 3H, J = 7.3 Hz, CH_3(Et)); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.4 (C-5); 160.9 (C-3); 159.8 (CO); 138.3 (C-4Ph); 134.7 (C-1Bn); 131.0 (C-1Ph); 129.7 (C-3Ph); 129.4 (C-2Ph); 128.7 (C-3Bn); 128.3 (C-4Bn); 127.3 (C-2Bn); 105.3 (C-4); 63.4 (CH_2(Et)); 52.1 (CH_2(Bn)); 14.0 (CH_3(Et)). MS (ES+) $[M+H]^+$ 405 (100%), $[M+H+2]^+$ 407 (42%). Anal. ($C_{19}H_{17}ClN_2O_4S$, 404.87): C, H, N, S.

5.2.13. 2-(2,4-Dichlorobenzyl)-3-(4-chlorophenyl)-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxylic acid ethyl ester (22). From 4-(4-chlorophenyl)-2,4-dioxobutyric acid ethyl ester **9** (0.363 g, 1.4 mmol) *N*-2,4-dichlorobenzylsulfamide **4** (0.364 g, 1.4 mmol) and dry ethanol (30 mL). Reaction time 120 h. Yield **22** (0.166 g of yellow oil, 24%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.38 (d, 2H, J = 8.5 Hz, *H*-2Ph); 7.24–7.22 (m, 3H, *H*s Bn); 7.24 (d, 2H, J = 8.5 Hz, *H*-3Ph); 6.77 (s, 1H, *H*-4); 5.06 (s, 2H, CH_2(Bn)); 4.44 (q, 2H, J = 7.3 Hz, CH_2(Et)); 1.42 (t, 3H, J = 7.3 Hz, CH_3(Et)). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 162.2 (C-5); 161.2 (C-3); 157.8 (CO); 138.7 (C-4Ph); 134.7 (C-1Bn); 133.0 (C-4Bn); 131.4 (C-2Bn); 130.6 (C-1Ph); 129.7 (C-3Ph); 129.3 (C-2Ph, C-6Bn); 129.4 (C-3Bn); 127.8 (C-5Bn); 105.4 (C-4); 63.5 (CH_2(Et)); 49.4 (CH_2(Bn)); 14.0 (CH_3(Et)). MS (ES+) $[M+H+Na]^+$ 495 (100%), $[M+H+2+Na]^+$ 497 (98%). Anal. ($C_{19}H_{15}Cl_3N_2O_4S$, 473.76): C, H, N, S.

5.2.14. 3-(4-Chlorophenyl)-2-hexyl-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxylic acid ethyl ester (23). From 4-(4-chlorophenyl)-2,4-dioxobutyric acid ethyl ester **9** (0.301 g, 0.012 mol), *N*-hexylsulfamide **6** (0.214 g, 0.012 mol) and dry ethanol (30 mL). Reaction time 96 h. Yield **23** (0.280 g, 59%); mp = oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.51 (d, 2H, J = 8.8 Hz; *H*-2Ph); 7.45 (d, 2H, J = 8.8 Hz; *H*-3Ph); 6.67 (s, 1H, *H*-4); 4.40 (q, 2H, J = 7.3 Hz, CH_2(Et)); 3.73 (m, 2H, *H*-1Hex); 1.61 (m, 2H, *H*-2Hex); 1.39 (t, 3H, J = 7.3 Hz, CH_3(Et)); 1.19 (m, 2H, *H*-5Hex); 1.13 (m, 2H, *H*-4Hex); 1.06 (m, 2H, *H*-3Hex); 0.78 (m, 3H, CH_3(Hex)). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 162.5 (C-5); 160.9 (C-3); 159.3 (CO); 138.3 (C-4Ph); 131.2 (C-1Ph); 129.7 (C-3Ph); 129.6 (C-2Ph); 104.2 (C-4); 63.3 (CH_2(Et)); 49.2 (C-1Hex); 30.8 and 30.6 (C-4 and C-2Hex); 25.9 (C-3Hex); 22.2 (C-5Hex); 14.0 (CH_3(Et)); 13.8 (CH_3). MS (ES+): $[M+H]^+$ 399 (100%); $[M+H+2]^+$ 401 (37%). Anal. ($C_{18}H_{23}ClN_2O_4S$, 398.9): C, H, N, S.

5.2.15. General procedure for the synthesis of thiadiazine-5-carboxamide 24–42. To a solution of the corresponding amine (0.5 mmol) in dry CH_2Cl_2 (2 mL), trimethylaluminum in hexane (0.5 mmol) was added dropwise under nitrogen atmosphere, and the mixture was stirred at room temperature for 1 h. Then, a solution of the corresponding 1,2,6-thiadiazine-5-carboxylic acid ethyl ester (0.1 mmol) in dry CH_2Cl_2 (2 mL) was added and refluxed for 24 h. After heating the mixture was treated with 2 N HCl and stirred at 40 °C for 30 min. The organic layer was separated, dried with sodium sulphate and evaporated to dryness. The residue was purified by silica gel column chromatography with CH_2Cl_2 as eluent to give the corresponding thiadiazine-5-carboxamide.

5.2.16. *N*-Cyclohexyl-2-phenyl-3-methyl-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxamide (24). From **11** (0.019 g, 0.06 mmol), *N*-1-cyclohexylamine (0.04 mL, d = 0.867, 0.3 mmol), 2 M $Al(CH_3)_3$ in hexane (0.15 mL, 0.3 mmol) in freshly distilled CH_2Cl_2 (4 mL). Reaction time 24 h. Yield **24** (0.019 g of brown solid, 86%); mp = 200–202 °C. ^{1}H NMR (300 MHz, $CDCl_3$)

δ: 7.54 (m, 3H, *H*-2, *H*-4Ph); 7.42 (m, 2H, *H*-3Ph); 6.82 (s, 1H, *H*-4); 3.84 (br m, 1H, *H*-1Cy); 2.07 (s, 3H, CH_3(3)); 1.94 (m, 2H, *H*-2eqCy); 1.75 (m, 2H, *H*-2ax(Cy)); 1.64 (m, 1H, *H*-4eq(Cy)); 1.40 (m, 1H, *H*-4ax(Cy)); 1.2–1.38 (m, 4H, H-3Cy). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 162.2 (C-5); 160.6 (C-3); 159.8 (CO); 133.5 (C-1Ph); 130.7 (C-4Ph); 130.1 (C-3Ph); 129.0 (C-2Ph); 98.5 (C-4); 49.0 (C-1Cy); 32.6 (C-2Cy); 25.3 (C-4Cy); 24.7 (C-3Cy); 22.3 (CH_3). MS (ES+): $[M+H]^+$ 348 (100%). Anal. ($C_{17}H_{21}N_3O_3S$, 347.43): C, H, N, S.

5.2.17. *N*-(Morpholin-1-yl)-2-benzyl-3-methyl-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxamide (25). From **12** (0.042 g, 0.14 mmol), *N*-1-aminomorpholine (0.08 mL, *d* = 1.059, 0.8 mmol), 2 M $Al(CH_3)_3$ in hexane (0.4 mL, 0.8 mmol) in freshly distilled CH_2Cl_2 (5 mL). Reaction time 24 h. Yield **25** (0.01 g of yellowish solid, 20%); mp = 155–157 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.15 (br s 1H, N*H*); 7.40–7.26 (m, 5H, *H*sPh); 6.74 (s, 1H, *H*-4); 5.16 (s, 2H, CH_2(Bn)); 3.82 (m, 4H, *H*-3Morf); 2.90 (m, 4H, *H*-2Morf); 2.27(s, 3H, CH_3(3)). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.2 (C-5); 159.7 (C-3); 158.1 (CO); 133.8 (C-1Bn); 129.3 (C-3Bn); 128.9 (C-4Bn); 126.7 (C-2Bn); 100.8 (C-4); 66.2 (C-2Morf); 55.7 (C-3Morf); 49.7 (CH_2(Bn)); 21.6 (CH_3(3)). MS (ES+): $[M+H]^+$ 365 (100%). Anal. ($C_{16}H_{20}N_4O_4S$, 364.42): C, H, N, S.

5.2.18. *N*-(Piperidin-1-yl)-2-benzyl-3-methyl-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxamide (26). From **12** (0.142 g, 0.47 mmol), *N*-1-aminopiperidine (0.25 mL, *d* = 0.928, 2.3 mmol), 2 M $Al(CH_3)_3$ in hexane (1.15 mL, 2.3 mmol) and freshly distilled CH_2Cl_2 (5 mL). Reaction time 24 h. Yield (0.037 g of yellow solid, 29%); mp = 85–87 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.09 (br s 1H, N*H*); 7.39–7.3 (m, 5H, *H*sPh); 6.70 (s, 1H, *H*-4); 5.16 (s, 2H, CH_2(Bn)); 2.80 (m, 4H, *H*-2Pip); 2.17 (s, 3H, CH_3(3)); 1.75 (m, 4H, *H*-3Pip); 1.45 (2H, m, *H*-4Pip). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.9 (C-5); 160.1 (C-3); 157.7 (CO); 134.0 (C-1Bn); 129.2 (C-3Ph); 128.4 (C-4Ph); 126.7 (C-2Ph); 100.9 (C-4); 56.9 (C-2Pip); 49.6 (CH_2(Bn)); 25.1 (C-3Pip); 23.1 (C-4Pip); 21.6 (CH_3(3)). MS (ES+): $[M+H]^+$ 363 (100%). Anal. ($C_{17}H_{22}N_4O_3S$, 362.45): C, H, N, S.

5.2.19. *N*-(Piperidin-1-yl)-2-(4-chlorophenyl)-3-methyl-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxamide (27). From **13** (0.037 g, 0.11 mmol), *N*-1-aminopiperidine (0.06 mL, *d* = 0.928, 0.56 mmol), 2 M $Al(CH_3)_3$ in hexane (0.27 mL, 0.56 mmol) in freshly distilled CH_2Cl_2 (4 mL). Reaction time 24 h. Yield **27** (0.01 g of yellow solid, 24%); mp = 185–187 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 10.8 (br s, 1H, N*H*); 7.51 (d, 2H, *J* = 8.8 Hz, *H*-3Ph); 7.46 (d, 2H, *J* = 8.8 Hz, *H*-2Ph); 5.06 (s, 1H, *H*-4); 2.85 (br m, 4H, *H*-2Pip); 2.40 (s, 3H, CH_3(3)); 1.74 (br m, 4H, *H*-3Pip); 1.54 (2H, br m, *H*-4Pip). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.6 (C-3); 158.3 (CO); 155.2 (C-5); 134.8 (C-1Ph); 129.9 (C-3Ph); 129.7 (C-4Ph); 126.9 (C-2Ph); 85.0 (C-4); 57.3 (C-2Pip); 25.0 (C-3Pip); 22.7 (C-4Pip); 19.4 (CH_3). MS (ES+): $[M+H]^+$ 383 (100%); $[M+H+2]^+$ 385 (38%). Anal. ($C_{16}H_{19}ClN_4O_3S$, 382.87): C, H, N, S.

5.2.20. *N*-(Piperidin-1-yl)-2-cyclohexyl-3-methyl-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxamide (28). From **14** (0.051 g, 0.16 mmol), *N*-aminopiperidine (0.09 mL, *d* = 0.928, 0.8 mmol), 2 M $Al(CH_3)_3$ in hexane (0.4 mL, 0.8 mmol) in freshly distilled CH_2Cl_2 (4 mL). Reaction time 24 h. Yield **28** (0.007 g of yellow solid, 13%); mp = 142–144 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.67 (br s 1H, N*H*); 5.49 (s, 1H, *H*-4); 4.01 (m, 1H, *H*-1ax(Cy)); 2.80 (m, 4H, *H*-2Pip); 2.32 (s, 3H, CH_3); 2.04 (m, 2H, *H*-2ax(Cy)); 1.99 (2H, m, *H*-2eq(Cy)); 1.85 (m, 2H, *H*-3ax(Cy)); 1.70 (m, 4H, *H*-3Pip); 1.48 (m, 2H, *H*-4Pip); 1.2–1.4 (m, 4H, *H*-3eq(Cy), *H*-4Cy). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.9 (C-3); 158.8 (CO); 155.8 (C-5); 84.0 (C-4); 57.3 (C-2Pip); 55.5 (C-1Cy); 29.8 (C-2Cy); 25.8 (C-3Cy); 25.0 (C-3Pip); 24.9 (C-4Cy); 22.8 (C-4Pip); 19.3 (CH_3). MS (ES+): $[M+H]^+$ 355 (100%). Anal. ($C_{16}H_{26}N_4O_3S$, 354.47): C, H, N, S.

5.2.21. *N*-(Piperidin-1-yl)-2-hexyl-3-methyl-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxamide (29). From **15a** (0.028 g, 0.09 mmol), *N*-aminopiperidine (0.05 mL, *d* = 0.928, 0.5 mmol), 2 M $Al(CH_3)_3$ in hexane (0.25 mL, 0.5 mmol) in freshly distilled CH_2Cl_2 (4 mL). Reaction time 24 h. Yield **29** (0.007 g of yellow solid, 22%); mp = 150–152 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.03 (br s 1H, N*H*); 6.70 (s, 1H, *H*-4); 3.87 (m, 2H, *H*-1Hex); 2.77 (m, 4H, *H*-1Pip); 2.40 (s, 3H, CH_3); 1.81 (m, 2H, *H*-2Hex); 1.73 (m, 4H, *H*-2Pip); 1.43 (m, 2H, *H*-3Pip); 1.36 (m, 2H, *H*-3Hex); 1.30 (m, 4H, *H*s Hex); 0.88 (m, 3H, CH_3(Hex)). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 161.9 (C-5); 159.8 (C-3); 157.8 (CO); 100.4 (C-4); 56.9 (C-1Pip); 46.9 (C-1Hex); 31.1; 30.5 (C-4Hex); 26.3 (C-3Hex); 25.1 (C-2Pip); 23.1 (C-3Pip); 22.4 (C-5Hex); 21.0 (CH_3); 13.9 (CH_3(Hex)). MS (ES+): $[M+H]^+$ 357(100%). Anal. ($C_{16}H_{28}N_4O_3S$, 356.48): C, H, N, S.

5.2.22. *N*-(Morpholin-1-yl)-2-benzyl-3-(4-bromophenyl)-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxamide (30). From **16** (0.050 g, 0.11 mmol), *N*-aminomorpholine (0.053 mL, *d* = 1.059, 0.55 mmol), 2 M $Al(CH_3)_3$ in hexane (0.27 mL, 0.55 mmol) in freshly distilled CH_2Cl_2 (5 mL). Yield **30** (0.038 g of yellow solid, 68%); mp = 199–201 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.19 (s, 1H, N*H*); 7.57 (d, 2H, *J* = 8.5 Hz, *H*-2Ph); 7.42–7.19 (m, 3H, *H*s Bn); 7.20 (d, 2H, *J* = 8.5 Hz, *H*-3Ph); 6.91 (s, 1H, *H*-4); 6.88 (m, 2H, *H*-2Bn); 5.02 (s, CH_2(Bn)); 3.86 (t, 4H, *J* = 4.4 Hz, *H*-3Morf); 2.94 (t, 4H, *J* = 4.4 Hz, *H*-2Morf). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 161.5 (C-5); 161.2 (C-3); 157.9 (CO); 134.7 (C-1Bn); 132.4 (C-3Ph); 131.5 (C-4Ph); 129.8 (C-2Ph); 128.8 (C-3Bn); 128.3 (C-4Bn); 127.2 (C-2Bn); 126.8 (C-1Ph); 104.1 (C-4); 66.2 (C-3Morf); 55.7 (C-2Morf); 52.1 (CH_2(Bn)). MS (ES+): $[M+H+2]^+$ 507 (100%), $[M+H]^+$ 505 (91%). Anal. ($C_{21}H_{21}BrN_4O_4S$, 505.38): C, H, N, S.

5.2.23. *N*-(Piperidin-1-yl)-2-benzyl-3-(4-bromophenyl)-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxamide (31). From **16** (0.037 g, 0.08 mmol), *N*-1-aminopiperidine (0.05 mL, *d* = 0.928, 0.45 mmol), 2 M $Al(CH_3)_3$ in hexane (0.22 mL, 0.45 mmol) in freshly distilled CH_2Cl_2

(4 mL). Reaction time 24 h. Yield **31** (0.027 g of yellow solid, 57%); mp = 194–196 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.1 (s, 1H, N*H*); 7.54 (d, 2H, J = 8.5 Hz, *H*-2Ph); 7.43–7.14 (m, 3H, *H*s Bn); 7.19 (d, 2H, J = 8.5 Hz, *H*-3Ph); 6.91 (s, 1H, *H*-4); 6.85 (dd, 2H, J = 7.1, 1.7 Hz, *H*-2Bn); 5.00 (s, CH_2(Bn)); 2.81 (m, 4H, *H*-2Pip); 1.74 (m, 4H, *H*-3Pip); 1.44 (m, 2H, *H*-4Pip). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 161.6 (C-5); 161.2 (C-3); 157.5 (CO); 134.7 (C-1Bn); 132.3 (C-3Ph); 131.5 (C-4Ph); 129.8 (C-2Ph); 128.7 (C-3Bn); 128.3 (C-4Bn); 127.2 (C-2Bn); 126.6 (C-1Ph); 104.1 (C-4); 56.8 (C-2Pip); 52.0 (CH_2(Bn)); 25.1 (C-3Pip); 23.1 (C-2Pip). MS (ES+): $[M+H]^+$ 503 (92%), $[M+H+2]^+$ 505 (100%). Anal. ($C_{22}H_{23}BrN_4O_3S$, 503.41): C, H, N, S.

5.2.24. *N*-(Piperidin-1-yl)-3-(4-bromophenyl)-2-cyclohexyl-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxamide (32). From **17** (0.008 g, 0.018 mmol), *N*-1-aminopiperidine (0.01 mL, d = 0.928, 0.09 mmol), 2 M $Al(CH_3)_3$ in hexane (0.04 mL, 0.09 mmol) in freshly distilled CH_2Cl_2 (3 mL). Reaction time 24 h. Yield **32** (0.004 g of yellow solid, 44%); mp = 85–87 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.06 (s, 1H, N*H*); 7.66 (d, 2H, J = 8.6 Hz, *H*-2Ph); 7.36 (d, 2H, J = 8.6 Hz, *H*-3Ph); 6.85 (s, 1H, *H*-4); 3.80 (m, 1H, *H*-1Cy); 2.80 (m, 2H, *H*-2Pip); 2.21 (m, 2H, *H*-2axCy); 2.01 (m, 2H, *H*-2eqCy); 1.84 (m, 2H, *H*-3axCy); 1.74 (m, 4H, *H*-3Pip); 1.13 (m, 2H, *H*-4Pip); 0.93–1.13 (m, 4H, *H*sCy). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 161.7 (C-5); 161.3 (C-3); 157.7 (CO); 135.2 (C-4Ph); 132.4 (C-3Ph); 129.4 (C-3Ph); 126.5 (C-1Ph); 104.1 (C-4); 65.2 (C-1Cy); 56.9 (C-2Pip); 31.6 (C-2Cy); 26.1 (C-3Cy); 25.1 (C-3Pip); 24.5 (C-4Cy); 23.1 (C-4Pip). MS (ES+): $[M+H]^+$ 495 (88%); $[M+H+2]^+$ 497 (100%); Anal. ($C_{21}H_{27}BrN_4O_3S$, 495.43): C, H, N, S.

5.2.25. *N*-(Piperidin-1-yl)-2-(2,4-dichlorobenzyl)-3-(4-bromophenyl)-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxamide (33). From **18** (0.065 g, 0.12 mmol), *N*-aminopiperidine (0.07 mL, d = 0.928, 0.6 mmol), 2 M $Al(CH_3)_3$ in hexane (0.3 mL, 0.6 mmol) in freshly distilled CH_2Cl_2 (4 mL). Reaction time 24 h. Yield **33** (0.046 g of orange solid, 65%,); mp = 215–217 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.1 (br s 1H, N*H*); 7.38 (d, 2H, J = 8.6 Hz, *H*-2Ph); 7.26 (d, 2H, J = 8.6 Hz, *H*-3Ph); 7.25 (m, 1H, *H*-5Bn); 7.22 (m, 1H, J = 2.2 Hz, *H*-3Bn); 7.19 (d, 1H, J = 8.8 Hz, *H*-6); 6.98 (s, 1H, *H*-4); 5.05 (s, 2H, CH_2(Bn)); 2.83 (m, 4H, *H*-2Pip); 1.76 (4H, m, *H*-3Pip); 1.46 (2H, m, *H*-4Pip). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 162.0 (C-5); 161.6 (C-3); 157.4 (CO); 134.8 (C-1Bn); 133.0 (C-2Bn); 132.6 (C-3Ph); 131.4 (C-4Bn); 131.1 (C-4Ph); 129.5 (C-2Ph and C-3Bn); 129.1 (C-3Bn); 127.8 (C-5Bn); 127.1 (C-1Ph); 104.1 (C-4); 56.9 (C-2Pip); 49.5 (CH_2(Bn)); 25.1 (C-3Pip); 23.1 (C-4Pip). MS (ES+): $[M+H]^+$ 573 (100%); 571 (61%) $[M+H+2]^+$ 575 (55%). Anal. ($C_{22}H_{21}BrCl_2N_4O_3S$, 572.3): C, H, N, S.

5.2.26. *N*-(1,3,3-Trimethylbicyclo[2.2.1]hept-2-yl)-2-benzyl-3-(4-chloro-3-methylphenyl)-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxamide (34). From **19** (0.037 g, 0.08 mmol), *N*-1,3,3-trimethylbicyclo[2.2.1]hept-2-ylamine hydrochloride (0.083 g, 0.5 mmol), which free-base was liberated by treatment with 20 mL of 5% NaOH and extraction with Et_2O (2× 20 mL), 2 M $Al(CH_3)_3$ in hexane (0.25 mL, 0.5 mmol) in freshly distilled CH_2Cl_2 (5 mL). Reaction time 24 h. Yield **34** (0.035 g of yellow solid, 76%); mp = 180–182 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.51 (da, 1H, J = 9.7 Hz, N*H*); 7.38 (d, 1H, J = 8.8 Hz, *H*-5Ph); 7.22 (m, 3H, *H*s Bn); 7.14 (m, 2H, *H*-6 and *H*-2 Ph); 6.93 (s, 1H, *H*-4); 6.91 (m, 2H, *H*-2Bn); 4.99 (s, 2H, CH_2-Bn); 3.66 (d, 1H, J = 9.7 Hz, *H*-1Bi); 2.29 (s, 3H, CH_3Ph); 1.81 (m, 1H, *H*-4Bi); 1.71 (m, 1H, *H*-5eqBi); 1.68 (m, 1H, *H*-7Bi); 1.50 (m, 1H, *H*-5axBi); 1.39 (m, 1H, *H*-6axBi); 1.28 (m, 1H, *H*-7Bi); 1.24 (m, 1H, *H*-6eqBi); 1.13 (s, 3H, CH_3(3)eqBi); 1.04 (s, 3H, CH_3(1)Bi); 0.84 (s, 3H, CH_3(3)axBi). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 161.8 (C-5); 161.4 (C-3); 161.3 (CO); 138.4 (C-3Ph); 137.4 (C-4Ph); 135.2 (C-1Bn); 131.2 (C-1Ph); 131.0 (C-2Ph); 129.8 (C-5Ph); 128.7 (C-3Bn); 128.2 (C-4Bn); 127.3 (C-2Bn); 127.0 (C-6Ph); 104.2 (C-4); 64.2 (C-1Bi); 52.5 (CH_2(Bn)); 48.8 (C-1Bi); 48.1 (C-4Bi); 42.7 (C-7Bi); 39.8 (C-3Bi); 30.9 (CH_3(3)eqBi); 27.1 (C-6Bi); 25.9 (C-5Bi); 21.2 (CH_3(3)axBi); 19.9 (CH_3Ph); 19.6 (CH_3(1)Bi). MS (ES+): $[M+H]^+$ 526 (100%); $[M+H+2]^+$ 528 (33%). Anal. ($C_{28}H_{32}ClN_3O_3S$, 526.09): C, H, N, S.

5.2.27. *N*-(Piperidin-1-yl)-2-benzyl-3-(4-chloro-3-methylphenyl)-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxamide (35). From **19** (0.056 g, 0.13 mmol), *N*-aminopiperidine (0.06 mL, d = 0.928, 0.6 mmol), 2 M $Al(CH_3)_3$ in hexane (0.25 mL, 0.6 mmol) in freshly distilled CH_2Cl_2 (5 mL). Reaction time 24 h. Yield **35** (0.034 g of yellow solid, 56%,); mp = 145–147 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.1 (br s 1H, N*H*); 7.38 (d, 1H, *H*-5Ph); 7.22 (m, 3H, *H*-3 and *H*-4 Bn); 7.12 (m, 2H, *H*-2 and *H*-6Ph); 6.90 (s, 1H, *H*-4); 6.88 (2H, m, *H*-2Bn); 4.98 (s, 2H, CH_2(Bn)); 2.80 (m, 4H, *H*-2Pip); 2.28 (s, 3H, CH_3-Ph); 1.74 (4H, m, *H*-3Pip); 1.45 (2H, m, *H*-4Pip). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 161.7. 161.6 (C-3 and C-5); 157.6 (CO); 138.4 (C-3Ph); 137.4 (C-4Ph); 135.1 (C-1Bn); 131.0 (C-1Ph); 130.9 (C-2Ph); 129.7 (C-5Ph); 128.7 (C-3Bn); 128.2 (C-4Bn); 127.2 (C-2Bn); 126.9 (C-6); 104.0 (C-4); 56.8 (C-2Pip); 52.3 (CH_2(Bn)); 25.1 (C-3Pip); 23.1 (C-4Pip); 19.9 (CH_3(Ph)). MS (ES+): $[M+H]^+$ 473 (100%); $[M+H+2]^+$ 475 (33%). Anal. ($C_{23}H_{25}ClN_4O_3S$, 472.99): C, H, N, S.

5.2.28. *N*-Cyclohexyl-2-(2,4-dichlorobenzyl)-3-(4-chloro-3-methylphenyl)-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxamide (36). From **20** (0.037 g, 0.08 mmol), *N*-cyclohexylamine (0.04 mL, d = 0.867, 0.4 mmol), 2 M $Al(CH_3)_3$ in hexane (0.2 mL, 0.4 mmol) in freshly distilled CH_2Cl_2 (5 mL). Reaction time 24 h. Yield **36** (0.028 g of yellow solid, 68%); mp = 147–149 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 10.4 (br s 1H, N*H*); 7.48 (d, 1H, J = 8.0 Hz, *H*-5Ph); 7.41 (d, 2H, J = 8.6 Hz, *H*-3Bn); 7.34 (d, 1H, J = 8.3 Hz, *H*-6Bn); 7.24 (d, 2H, J = 1.8 Hz, *H*-2Ph); 7.22 (dd, 1H, J = 8.3, 2.2 Hz, *H*-5Bn); 7.13 (dd, 1H, J = 8.0, 1.8 Hz, *H*-6Ph); 5.67 (s, 1H, *H*-4); 4.95 (s, 2H, CH_2(Bn)); 3.56 (m, 1H, *H*-1axCy); 2.45 (s, 3H, CH_3-Ph); 1.88 (m, 2H, *H*-2eqCy); 1.78 (m, 2H, *H*-3eqCy); 1.54 (m, 3H, *H*-2ax, *H*-4eqCy); 1.28 (m, 3H, *H*-3ax, *H*-4axCy). ^{13}C NMR (125 MHz,

$CDCl_3$) δ: 169.1 (C-3); 159.4 (CO); 155.9 (C-5); 137.7. 137.6 (C-3, C-4Ph); 134.5, 134.0 (C-1, C-2Bn); 131.9 (C-1(Ph)); 130.4 (C-4Bn); 130.2 (C-6Bn); 129.8 (C-5Ph); 129.5 (C-2Ph); 129.4 (C-3Bn); 127.4 (C-5Bn); 125.6 (C-6Ph); 87.6 (C-4); 55.6 (C-1Cy); 41.5 (CH_2(Bn)); 33.8 (C-2Cy); 24.7 (C-4Cy); 23.9 (C-3Cy); 20.2 (CH_3-Ph). MS (ES+): $[M+H]^+$ 540 (91%); $[M+H+2]^+$ 542 (100%); $[M+H+4]^+$ 544 (35%). Anal. ($C_{24}H_{24}Cl_3N_3O_3S$, 540.89): C, H, N, S.

5.2.29. *N*-Phenyl-2-benzyl-3-(4-chlorophenyl)-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxamide (37). From **21** (0.063 g, 0.15 mmol), aniline (0.07 mL, d = 1.022, 0.74 mmol), 2 M $Al(CH_3)_3$ in hexane (0.37 mL, 0.74 mmol) in freshly distilled CH_2Cl_2 (10 mL). Reaction time 24 h. Yield **37** (0.054 g of yellow solid, recrystallised in MeOH, 80%); mp = 187–189 °C (MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.21 (br s 1H, N*H*); 7.69 (m, 2H, *H*-2Ph); 7.42–7.29 (m, 10H, *H*s Bn, *H*-3Ph and N*H*-Ph); 7.03 (s, 1H, *H*-4); 6.87 (2H, m, *H*-2Bn); 5.07 (s, 2H, CH_2(Bn)). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 161.6 (C-5); 161.5 (C-3); 158.3 (CO); 138.5 (C-4Ph); 136.4 (C-1NH-Ph); 134.7 (C-1Bn); 131.1 (C-1Ph); 129.8 (C-3Ph); 129.5 (C-2Ph); 129.3 (C-3NH-Ph); 128.8 (C-3Bn); 128.4 (C-4Bn); 127.2 (C-2Bn); 125.4 (C-4NH-Ph); 119.9 (C-2NH-Ph); 104.1 (C-4); 52.2 (CH_2(Bn)). MS (ES+): $[M+H]^+$ 452 (100%); $[M+H+2]^+$ 454 (33%). Anal. ($C_{23}H_{18}ClN_3O_3S$, 451.93): C, H, N, S.

5.2.30. *N*-(Piperidin-1-yl)-2-benzyl-3-(4-chlorophenyl)-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxamide (38). From **21** (0.151 g, 0.37 mmol), *N*-aminopiperidine (0.2 mL, d = 0.928, 1.85 mmol), 2 M $Al(CH_3)_3$ in hexane (0.92 mL, 1.85 mmol) in freshly distilled CH_2Cl_2 (5 mL). Reaction time 24 h. Yield **38** (0.125 g of yellow solid, 74%); mp = 184–186 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.01 (br s 1H, N*H*); 7.40 (m, 2H, *H*-2Ph); 7.30 (m, 2H, *H*-3Ph); 7.23–7.17 (m, 3H, *H*s Bn); 6.91 (s, 1H, *H*-4); 6.87 (2H, m, *H*-2Bn); 5.01 (s, 2H, CH_2(Bn)); 2.82 (m, 4H, *H*-2Pip); 1.73 (m, 4H, *H*-3Pip); 1.47 (m, 2H, *H*-4Pip). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 161.6 (C-5); 161.2 (C-3); 157.5 (CO); 138.3 (C-4Ph); 134.8 (C-1Bn); 131.1 (C-1Ph); 129.8 (C-3Ph); 129.4 (C-2Ph); 128.8 (C-3Bn); 128.3 (C-4Bn); 127.2 (C-2Bn); 104.2 (C-4); 56.9 (C-2Pip); 52.1 (CH_2(Bn)); 25.1 (C-3Pip); 23.1 (C-4Pip). MS (ES+): $[M+H]^+$ 459 (100%); $[M+H+2]^+$ 461 (33%). Anal. ($C_{22}H_{23}ClN_4O_3S$, 458.12): C, H, N, S.

5.2.31. *N*-(1,3,3-Trimethylbicyclo[2.2.1]hept-2-yl)-2-benzyl-3-(4-chlorophenyl)-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxamide (39). From **21** (0.047 g, 0.12 mmol), *N*-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl-amine hydrochloride (0.141 g, 0.92 mmol), which freebase was liberated by treatment with 20 mL of 5% NaOH and extraction with Et_2O (2× 20 mL), 2 M $Al(CH_3)_3$ in hexane (0.46 mL, 0.92 mmol) in CH_2Cl_2 (4 mL) freshly distilled. Reaction time 24 h. Yield **32** (0.019 g of yellow solid, 88%); mp = 160–162 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.48 (da, 1H, J = 9.0 Hz, N*H*); 7.40 (d, 2H, J = 8.7 Hz, *H*-2Ph); 7.30 (d, 2H, J = 8.7 Hz, *H*-3Ph); 7.20 (m, 3H, *H*-3 and *H*-4Bn); 6.86 (d, 2H, J = 7.0 Hz, *H*-2Bn); 6.92 (s, 1H, *H*-4); 5.00 (s, 2H, CH_2-Bn); 3.65 (d, 1H, J = 9.0 Hz, *H*-1Bi); 1.81 (m, 1H, *H*-4Bi); 1.74 (m, 1H, *H*-5eqBi); 1.70 (m, 1H, *H*-7Bi); 1.52 (m, 1H, *H*-5axBi); 1.42 (m, 1H, *H*-6axBi); 1.28 (m, 1H, *H*-7Bi); 1.25 (m, 1H, *H*-6eqBi); 1.14 (s, 3H, CH_3(3)eqBi); 1.08 (s, 3H, CH_3(1)Bi); 0.84 (s, 3H, CH_3(3)axBi). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 161.8 (C-5); 161.3 (C-3); 160.9 (CO); 138.3 (C-4Ph); 134.9 (C-1Bn); 131.2 (C-1Ph); 129.8 (C-3Ph); 129.5 (C-2Ph); 128.8 (C-3Bn); 128.3 (C-4Bn); 127.3 (C-2Bn); 104.4 (C-4); 52.3 (CH_2(Bn)); 64.2 (C-2Bi); 48.8 (C-1Bi); 48.1 (C-4Bi); 42.7 (C-7Bi); 39.8 (C-3Bi); 31.0 (C-1Bi); 30.9 (CH_3(3)eqBi); 27.2 (C-6Bi); 25.9 (C-5Bi); 21.2 (CH_3(3)axBi); 19.6 (CH_3(1)Bi). MS (ES+): $[M+H]^+$ 512 (100%); $[M+H+2]^+$ 514 (33%). Anal. ($C_{27}H_{30}ClN_3O_3S$, 512.06): C, H, N, S.

5.2.32. *N*-(1,3,3-Trimethylbicyclo[2.2.1]hept-2-yl)-2-(2,4-dichlorobenzyl)-3-(4-chlorophenyl)-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxamide (40). From **22** (0.040 g, 0.08 mmol), *N*-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl-amine hydrochloride (0.126 g, 0.5 mmol), which freebase was liberated by treatment with 20 mL of 5% NaOH and extraction with Et_2O (2× 20 mL), 2 M $Al(CH_3)_3$ in hexane (0.33 mL, 0.6 mmol) in freshly distilled CH_2Cl_2 (5 mL). Reaction time 24 h. Yield **40** (0.025 g of yellow solid, 54%); mp = 65–67 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.5 (da, 1H, J = 9.8 Hz, N*H*); 7.37 (d, 1H, J = 8.0 Hz, *H*-2Ph); 7.27 (d, 1H, J = 8.0 Hz, *H*-3Ph); 7.26-7.21 (m, 3H, *H*s Bn); 6.99 (s, 1H, *H*-4); 5.05 (s, 2H, CH_2-Bn); 3.68 (dd, 1H, J = 9.8, 1.95 Hz, *H*-2Bi); 1.81 (d, 1H, J = 3.4 Hz, *H*-4Bi); 1.74 (m, 1H, *H*-5eqBi); 1.70 (m, 1H, *H*-7Bi); 1.52 (m, 1H, *H*-5axBi); 1.40 (m, 1H, *H*-6axBi); 1.28 (m, 1H, *H*-7Bi); 1.22 (m, 1H, *H*-6eqBi); 1.14 (s, 3H, CH_3(3)eqBi); 1.09 (s, 3H, CH_3(1)Bi); 0.85 (s, 3H, CH_3(3)axBi). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 161.3 (C-5); 161.2 (C-3); 161.1 (CO); 138.4 (C-4Ph); 134.7 (C-1Bn); 132.9 (C-2Bn); 131.6 (C-4Bn); 130.8 (C-1Ph); 129.6 (C-3(Ph)); 129.5 (C-2Ph and C-3Bn); 129.2 (C-2Bn); 127.8 (C-5Bn); 104.3 (C-4); 64.3 (C-2Bi); 49.7 (CH_2-Bn); 48.8 (C-1Bi); 48.1 (C-4Bi); 42.7 (C-7Bi); 39.8 (C-3Bi); 30.1 (CH_3(3)eq-Bi); 27.2 (C-6Bi); 25.9 (C-5Bi); 21.3 (CH_3(3)axBi); 19.7 (CH_3(1)Bi). MS (ES+): $[M+H]^+$ 580 (97%); $[M+H+2]^+$ 582 (100%); $[M+H+4]^+$ 584 (37%). Anal. ($C_{27}H_{28}Cl_3N_3O_3S$, 580.95): C, H, N, S.

5.2.33. *N*-(Piperidin-1-yl)-2-(2,4-dichlorobenzyl)-3-(4-chlorophenyl)-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxamide (41). From **22** (0.062 g, 0.12 mmol), *N*-aminopiperidine (0.071 g, d = 0.928, 0.6 mmol), 2 M $Al(CH_3)_3$ in hexane (0.3 mL, 0.6 mmol) in freshly distilled CH_2Cl_2 (4 mL). Reaction time 24 h. Yield **33** (0.027 g of orange solid, 43%); mp = 208–210 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.1 (br s, 1H, N*H*); 7.38 (d, 2H, J = 8.6 Hz, *H*-2Ph); 7.26 (d, 2H, J = 8.6 Hz, *H*-3Ph); 7.25 (m, 1H, *H*-5Bn); 7.22 (m, 1H, J = 2.2 Hz *H*-3Bn); 7.19 (d, 1H, J = 8.8 Hz, *H*-6Bn); 6.98 (s, 1H, *H*-4); 5.05 (s, 2H, CH_2(Bn)); 2.83 (m, 4H, *H*-2Pip); 1.76 (4H, m, *H*-3Pip); 1.46 (2H, m, *H*-4Pip). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 161.9 (C-5); 161.5 (C-3); 157.4 (CO); 138.7 (C-4Ph); 134.7 (C-1Bn); 132.9 (C-2(Bn)); 131.4 (C-1Ph); 130.6 (C-4Bn); 129.7 (C-3Ph); 129.5 (C-6Bn); 129.4 (C-2Ph); 129.1 (C-3Bn); 127.8 (C-5Bn);

104.2 (C-4); 56.9 (C-2Pip); 49.5 (CH_2(Bn)); 25.1 (C-3Pip); 23.1 (C-4Pip). MS (ES+): $[M+H]^+$ 527 (95%); $[M+H+2^+]$ 529 (100%); $[M+H+4]^+$ 531 (39%). Anal. ($C_{22}H_{21}Cl_3N_4O_3S$, 527.85): C, H, N, S.

5.2.34. *N*-(Piperidin-1-yl)-3-(4-chlorophenyl)-2-hexyl-1,1-dioxo-1,2-dihydro-1$^{\lambda 6}$-1,2,6-thiadiazine-5-carboxamide (42). From **23** (0.047 g, 0.12 mmol), *N*-aminopiperidine (0.06 mL, $d = 0.928$, 0.6 mmol), 2 M $Al(CH_3)_3$ in hexane (0.29 mL, 0.6 mmol) in freshly distilled CH_2Cl_2 (4 mL). Reaction time 5 h. Yield **42** (0.035 g of yellow solid, 65%); mp = 132–134 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.08 (s, 1H, N*H*); 7.49 (d, 2H, $J = 8.8$ Hz; *H*-2Ph); 7.46 (d, 2H, $J = 8.8$ Hz; *H*-3Ph); 6.87 (s, 1H, *H*-4); 3.74 (m, 2H, *H*-2Hex); 2.79 (m, 4H, *H*-2Pip); 1.74 (m, 4H, *H*-3Pip); 1.60 (m, 2H, *H*-2Hex); 1.44 (m, 2H, *H*-4Pip); 1.08 (m, 6H, *H*s Hex); 0.78 (m, 3H, C*H*$_3$(Hex)). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 161.2 (C-5); 161.1 (C-3); 157.6 (CO); 138.3 (C-4Ph); 131.2 (C-1Ph); 129.7 (C-3Ph); 129.6 (C-2Ph); 103.1 (C-4); 49.2 (C-1Hex); 30.8 (C-4Hex); 30.5 (C-2Hex); 25.8 (C-3Hex); 25.1 (C-3Pip); 23.1 (C-4Pip); 22.2 (C-5Hex); 13.8 (CH_3). MS (ES+): $[M+H]^+$ 453 (100%); $[M+H+2]^+$ 455 (37%). Anal. ($C_{21}H_{29}ClN_4O_3S$, 452.16): C, H, N, S.

5.3. Pharmacological assays

5.3.1. Contractile responses in isolated tissue. For this study, male ICR mice weighing 25–30 g were used. Mouse vas deferens (MVD) were isolated as described by Hughes.[56] Tissues were suspended in a 10 mL organ bath containing 5 mL of Krebs solution (NaCl 118; KCl 4.75; $CaCl_2$ 2.54; KH_2PO_4 1.19; $MgSO_4$ 1.2; $NaHCO_3$ 25; glucose 11 mM) that was continuously gassed with 95% O_2 and 5% CO_2. Tissues were kept under 0.5 g of resting tension at 37 °C and were electrically stimulated through two platinum ring electrodes. They were subjected to alternate periods of stimulation (trains of five rectangular pulses of 70 V, 15 Hz and 2 ms duration each were applied every minute) and rest (10 min). The isometric force was monitored by computer using a MacLab data recording and analysis system.

(1) Agonistic activity: The effect of the synthetic cannabinoid agonist WIN 55,212-2 (10^{-7}–8.1×10^{-6} M) and of the new compounds **24**–**42** (10^{-7}–2.4×10^{-5} M) was tested by constructing concentration–response curves for them in a step by step manner. Curves were carried out by the following protocol: WIN 55,212-2 or the new compounds were added at a dose to the organ bath 50 min after the beginning of electrical stimulation and their effect on the electrically induced contractions was evaluated 10 min after their addition. Then, the electrical stimulation was stopped, Krebs solution was replaced and the following dose of the compounds was added. This protocol was repeated for every dose.

Results have been expressed as % of inhibition, taking the mean amplitude of the last five contractions before the addition of the first dose of compounds as 100%. Each tissue was employed only once.

(2) Antagonistic activity: To test antagonistic activity of the new compounds lacking agonistic activity (**24**–**28**, **30**, **31**, **33**–**35**, **38**, **40**–**42**), the effect of WIN 55,212-2 (10^{-7}–8.1×10^{-6} M) was tested in: control tissues (control values), and after incubation with new compounds (10^{-6} M) or with the reference cannabinoid antagonist SR141716A (10^{-6} M). Concentration–response curves for the reference cannabinoid receptor agonist were constructed in a step by step manner as follows:

SR141716A or the new compounds (**24**–**28**, **30**, **31**, **33**–**35**, **38**, **40**–**42**) were added to the organ bath 50 min after the beginning of electrical stimulation and 10 minutes later, a dose of WIN 55,212-2 was added and its effect on the electrically induced contractions was tested 10 min later. Then, the electrical stimulation was stopped, Krebs solution was replaced and the new compounds or SR141716A were added again to test the effect of the following concentration of the agonist. This protocol was repeated for every dose of the cannabinoid reference agonist.

Results have been expressed as % of inhibition, taking the mean amplitude of the last five contractions before the first addition of the agonist as 100%. Each tissue was employed to construct only one concentration–response curve.

5.3.2. In vivo bioassays. ICR male mice weighing 25–30 g were used. Spontaneous behaviour was always observed in the cage before treatment and/or performance of the different tests. Animals showing spontaneous behavioural modifications were discarded.

WIN 55,212-2 and new compounds **37**, **39** and **38** were administered 15 min before starting the cannabinoid tetrad of behavioral tests to evaluate their agonistic effects and when compound **38** or SR141716A were tested as antagonists they were administered 20 min before the reference agonist.

5.3.2.1. Cannabinoid tetrad. Separated groups of mice ($n = 8$–12) were given one of the following treatments:

ip administration: (1) saline solution or vehicle (control groups), (2) WIN 55,212-2 (1.5 mg/kg), (3) **37** (10 mg/kg), (4) **37** (20 mg/kg), (5) **39** (10 mg/kg), (6) **39** (20 mg/kg), (7) **38** (1 mg/kg), (8) **38** (1 mg/kg) + WIN 55,212-2 (1.5 mg/kg), (9) **38** (5 mg/kg) + WIN 55,212-2 (1.5 mg/kg), (10) **38** (10 mg/kg) + WIN 55,212-2 (1.5 mg/kg) and (11) SR141716A (1 mg/kg) + WIN 55,212-2 (1.5 mg/kg).

icv administration: (1) vehicle (control group), (2) WIN 55,212-2 (0.1 mg/kg), (3) WIN 55,212-2 (0.2 mg/kg), (4) WIN 55,212-2 (0.4 mg/kg), (5) **37** (0.2 mg/kg), (6) **37** (0.4 mg/kg), (7) **37** (0.8 mg/kg), (8) **39** (0.2 mg/kg), (9) **38** (0.05 mg/kg), (10) vehicle + WIN 55,212-2 (1.5 mg/kg, ip), (11) **38** (0.05 mg/kg) + WIN 55,212-2 (1.5 mg/kg, ip) and (12) **38** (0.1 mg/kg) + WIN 55,212-2 (1.5 mg/kg, ip).

In the sham group, mice were subjected to the same procedure than icv given drugs groups.

Tests were consecutively conducted with 5 min of interval between them by following:

(1.1) *Hypothermia*: Core temperatures in mice were measured using a lubricated thermometer inserted into the rectum to a constant depth of 1 cm. Temperature was evaluated twice in each animal: before and after every treatment.

(1.2) *Nociception*: The hot plate test was carried out using a hot plate at 55 °C as nociceptive stimulus. The latency time of licking of the front paw was taken as an index of nociception. The latency was measured before treatment (control latency) and after every treatment (latency after treatment). The cut-off time was 30 s and analgesia was quantified with the formula of the Maximum Possible Effect (M.P.E.), expressed as a percentage:

$$\%\ \text{M.P.E.} = (\text{Latency after treatment} - \text{control latency})/(\text{cut-off time} - \text{control latency}) \times 100.$$

(1.3) *Catalepsy*: Catalepsy was measured using a modified 'ring test' as originally described by Pertwee.[57] Mice were placed on a rubber coated metal ring (6 cm diameter) fixed horizontally at a height of 30 cm. CB_1 cannabinoid agonists cause animals to become cataleptic, the sum of all times during the mice were immobile was registered for a 5 min period and compared with the time registered in control animals. The criterion for immobility was the absence of all voluntary movements. Catalepsy was evaluated once in each animal after every treatment.

(1.4) *Locomotor activity*: Spontaneous locomotor activity was evaluated using individual photocell activity chambers (Cibertec®). Mouse was placed in a chamber and, starting 10 min later, the number of interruptions of photocell beams was recorded over a 30-min period. The mean number of crossing has been compared with that obtained from mice control groups. This parameter was evaluated once in each animal after every treatment.

5.4. Data analysis

Data are expressed as means ± standard error of the mean (SEM). For the in vitro assays, values for EC_{50} and 95% confidence limits of these values for agonists, were calculated by nonlinear regression analysis using the equation for a sigmoid concentration–response curve (GraphPad Prism).[43] The pA_2 value for each compound, as defined by Arunlakshana and Schild,[58] was obtained from a linear regression plot of the mean values obtained for log(CR-1) versus the negative logarithm of the antagonist concentration (CR being the concentration ratio of the agonist in the presence or absence of antagonist).[59] For the effects of drugs on in vitro and in vivo tests, one- or two-way analysis of variance (ANOVA) tests have been used for the statistical analysis of multiple comparisons. When a significant difference was detected the data were further analyzed using Bonferroni's test. In each test, a P value less than 0.05 was considered to indicate statistical significance.

To compare the EC_{50} of WIN 55,212-2 obtained from control group or incubated with compounds **38**, **42** or SR141716A groups, in the in vitro assays, a two-tailed unpaired Student's t-test (confidence intervals 99%) that compared means differences was used.

5.5. Drugs

WIN 55,212-2 mesylate and SR141716A were obtained from TOCRIS (Biogen Científica S.L., Madrid, Spain) and from SANOFI AVENTIS (Paris, France), respectively.

Cannabinoids were dissolved in ethanol 1 mg:1 mL and subsequently in ethanol and Tween 80 (1:2) after which the ethanol was evaporated[25] and saline solution (0.9%) was added.

Acknowledgment

This project is supported by the Ministerio de Ciencia y Tecnologia, SPAIN SAF2003-08003-C02 and SAF2006-13391-C03.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.07.056.

References and notes

1. Gaoni, Y.; Mechoulam, R. *J. Am. Chem. Soc.* **1964**, *86*, 1646–1647.
2. Thakur, G. A.; Duclos, R. I., Jr.; Makriyannis, A. *Life Sci.* **2005**, *78*, 454–466.
3. Thakur, G. A.; Nikas, S. P.; Makriyannis, A. *Mini Rev. Med. Chem.* **2005**, *5*, 631–640.
4. Devane, W. A.; Hanus, L.; Breuer, A.; Pertwee, R. G.; Stevenson, L. A.; Grin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. *Science* **1992**, *258*, 1946–1949.
5. Di Marzo, V. *Trends Pharmacol. Sci.* **2006**, *27*, 134–140.
6. Smith, R. A.; Fathi, Z. *IDrugs* **2005**, *8*, 53–66.
7. Pertwee, R. G. *Handb. Exp. Pharmacol.* **2005**, *168*, 1–51.
8. Pertwee, R. G. *Life Sci.* **2005**, *76*, 1307–1324.
9. Padgett, L. W. *Life Sci.* **2005**, *77*, 1767–1798.
10. Huffman, J. W. *Mini Rev. Med. Chem.* **2005**, *5*, 641–649.
11. Matsuda, L. A.; Lolait, S. J.; Brownstein, M. J.; Young, A. C.; Bonner, T. I. *Nature* **1990**, *346*, 561–564.
12. Munro, S.; Thomas, K. L.; Abu-Shaar, M. *Nature* **1993**, *365*, 61–65.
13. Begg, M.; Pacher, P.; Batkai, S.; Osei-Hyiaman, D.; Offertaler, L., et al. *Pharmacol. Ther.* **2005**, *106*, 133–145.
14. Baker, D.; Pryce, G.; Davies, W. L.; Hiley, C. R. *Trends Pharmacol. Sci.* **2006**, *27*, 1–4.
15. Brown, A. J.; Wise, A. Glaxosmithkline. Identification of modulators of GPR55 activity. WO0186305. 2001.
16. Drmota, T. G., Peter; Groblewski, Thierry. Astrazeneca. Screening assays for cannabinoid-ligand type modulators of GPR55. Ge: PCT Int. Appl. WO 2004074844 A1 2 Sep 2004, 2004.

17. Patny, A.; Desai, P. V.; Avery, M. A. *Curr. Med. Chem.* **2006**, *13*, 1667–1691.
18. Montero, C.; Campillo, N. E.; Goya, P.; Paez, J. A. *Eur. J. Med. Chem.* **2005**, *40*, 75–83.
19. Bifulco, M.; Laezza, C.; Pisanti, S.; Gazzerro, P. *Br. J. Pharmacol.* **2006**, *148*, 123–135.
20. Hall, W.; Christie, M.; Currow, D. *Lancet Oncol.* **2005**, *6*, 35–42.
21. Barnes, M. P. *Exp. Opin. Pharmacother.* **2006**, *7*, 607–615.
22. Carai, M. A.; Colombo, G.; Gessa, G. L. *Life Sci.* **2005**, *31*, 31.
23. Goya, P.; Jagerovic, N.; Hernandez-Folgado, L.; Martin, M. I. *Mini Rev. Med. Chem.* **2003**, *3*, 765–772.
24. Elguero, J.; Ochoa, C.; Stud, M.; Esteban Calderón, C.; Martínez Ripoll, M.; Fayet, J. P.; Vertut, M. C. *J. Org. Chem.* **1982**, *47*, 536–544.
25. Pertwee, R. G.; Stevenson, L. A.; Elrick, D. B.; Mechoulam, R.; Corbett, A. D. *Br. J. Pharmacol.* **1992**, *105*, 980–984.
26. Martin, B. R.; Compton, D. R.; Thomas, B. F.; Prescott, W. R.; Little, P. J.; Razdan, R. K.; Johnson, M. R.; Melvin, L. S.; Mechoulam, R.; Ward, S. J. *Pharmacol. Biochem. Behav.* **1991**, *40*, 471–478.
27. Wiley, J. L.; Martin, B. R. *Eur. J. Pharmacol* **2003**, *471*, 185–193.
28. Páez, J. A.; Goya, P.; Cano, C.; Martín, M. I.; Girón, M. R; Goicoechea, C. Derivados de 1,1-dioxo-1,2,6-tiadiazina-5-carboxamida de estructura química I y de 1,1-dioxo-1,2,6-tiadiazina-5-carboxilato de alquilo de estructura química II, con propiedades cannabinoides. Pat. Esp. 200502759, 2005.
29. Wright, J. B. *J. Org. Chem.* **1964**, *29*, 1905–1909.
30. Wright, J. B. 3-Carbalkoxy and 3-carbamoyl-5-substituted-2*H*-1,2,6-thiadiazine-1,1-dioxides and process. Patent, Upjohn Co. U.S. 3223703, 1963; *Chem. Abstr.* **1966**, *64*, 8218.
31. Lange, J. H.; Kruse, C. G. *Drug Discov. Today* **2005**, *10*, 693–702.
32. Chen, J. Z.; Han, X. W.; Liu, Q.; Makriyannis, A.; Wang, J.; Xie, X. G. *J. Med. Chem.* **2006**, *26*, 625–636.
33. Paquin, A. M. *Angew. Chem.-Ger. Ed.* **1948**, *60*, 316–320.
34. Houlihan, W. J. Chlorobenzylsulfamides. U.S. Patent Application 3,320,314, 1967.
35. Mcdermott, S. D.; Spillane, W. J. *Synthesis-Stuttgart* **1983**, 192–195.
36. Lee, C. H.; Kohn, H. *J. Org. Chem.* **1990**, *55*, 6098–6104.
37. Graf, R. *Chem. Ber-Recl.* **1959**, *92*, 509–513.
38. Iwanami, Y.; Seki, T. *Bull. Chem. Soc. Jpn.* **1972**, *45*, 2829–2834.
39. Gardner, T. S.; Lee, J.; Wenis, E. *J. Org. Chem.* **1961**, *26*, 1514–1518.
40. Seltzman, H. H.; Foster, M. C.; Wyrick, C. D.; Burgess, J. P.; Carroll, F. I. *J. Label. Compd. Radiopharm.* **2005**, *48*, 589–596.
41. Benderly, A.; Stavchansky, S. *Tetrahedron Lett.* **1988**, *29*, 739–740.
42. Basha, A.; Lipton, M.; Weinreb, S. M. *Tetrahedron Lett.* **1977**, 4171–4174.
43. Thomas, A.; Pertwee, R. G. *Methods Mol. Med.* **2006**, *123*, 191–207.
44. Pertwee, R.; Griffin, G.; Fernando, S.; Li, X.; Hill, A.; Makriyannis, A. *Life Sci.* **1995**, *56*, 1949–1955.
45. Pertwee, R.; Griffin, G.; Lainton, J. A.; Huffman, J. W. *Eur. J. Pharmacol.* **1995**, *284*, 241–247.
46. Ross, R. A.; Gibson, T. M.; Stevenson, L. A.; Saha, B.; Crocker, P.; Razdan, R. K.; Pertwee, R. G. *Br. J. Pharmacol.* **1999**, *128*, 735–743.
47. Schlicker, E.; Redmer, A.; Werner, A.; Kathmann, M. *Br. J. Pharmacol.* **2003**, *140*, 323–328.
48. Jagerovic, N.; Hernandez-Folgado, L.; Alkorta, I.; Goya, P.; Martin, M. I.; Dannert, M. T.; Alsasua, A.; Frigola, J.; Cuberes, M. R.; Dordal, A.; Holenz, J. *Eur. J. Med. Chem.* **2006**, *41*, 114–120.
49. Colombo, G.; Agabio, R.; Lobina, C.; Reali, R.; Gessa, G. L. *Eur. J. Pharmacol.* **1998**, *344*, 67–69.
50. Gessa, G. L.; Casu, M. A.; Carta, G.; Mascia, M. S. *Eur. J. Pharmacol.* **1998**, *355*, 119–124.
51. Sim-Selley, L. J.; Brunk, L. K.; Selley, D. E. *Eur. J. Pharmacol.* **2001**, *414*, 135–143.
52. Mato, S.; Pazos, A.; Valdizan, E. M. *Eur. J. Pharmacol.* **2002**, *443*, 43–46.
53. Savinainen, J. R.; Saario, S. M.; Niemi, R.; Jarvinen, T.; Laitinen, J. T. *Br. J. Pharmacol.* **2003**, *140*, 1451–1459.
54. Fride, E.; Perchuk, A.; Hall, F. S.; Uhl, G. R.; Onaivi, E. S. *Methods Mol. Med.* **2006**, *123*, 269–290.
55. Martin, B. R.; Thomas, B. F.; Razdan, R. K. Structural Requirements for Cannabinoid Receptor Probes. In *Cannabinoid Receptors*; Pertwee, R. G., Ed.; Academic Press: London, 1995; pp 35–86.
56. Hughes, J.; Kosterlitz, H. W.; Leslie, F. M. *Br. J. Pharmacol.* **1975**, *53*, 371–381.
57. Pertwee, R. G. *Br. J. Pharmacol.* **1972**, *46*, 753–763.
58. Arunlakshana, O.; Schild, H. O. *Br. J. Pharmacol. Chemother.* **1959**, *14*, 48–58.
59. Maruyama, I.; Tomiyama, Y.; Maruyama, K.; Ojima, K.; Kobayashi, K.; Kobayashi, M.; Yamazaki, Y.; Kojima, M.; Shibata, N. *Eur. J. Pharmacol.* **2006**, *531*, 194–200.